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Reconstitution of an electrically active conformational transition in rhodopsin-containing membranes

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An electrically active event that has been observed in native rod outer segment disk membranes can be reconstituted into membrane vesicles containing purified rhodopsin and defined phospholipids. The magnitude of this charge-transfer event, as estimated using spin-labeled derivatives of hydrophobic ions, is a function of the phospholipid composition. In reconstituted membranes containing rhodopsin and egg phosphatidylcholine, the charge transferred during this event is approximately 10% that measured in the native system. The addition of 20 mol% egg phosphatidylethanolamine, phosphatidic acid or brain phosphatidylserine returns the magnitude of the charge transfer to within 60 to 100% of the native activity. The response seen in the reconstituted membrane system is consistent with a previously proposed interfacial charge-transfer mechanism.

The earliest molecular events responsible for the ionic and biochemical changes observed in the vertebrate rod photoreceptor occur in rhodopsin, a membrane protein of the rod outer segment disk membrane. Little is known, however, of the structural changes that occur in rhodopsin as a result of the absorption of light by the retinal chromophore. In particular, the structural changes that result in the activation of the rod cell enzymology or alteration of the ion distribution in the rod cell have not been elucidated. One conformational transition that occurs in bovine rhodopsin upon photolysis has been shown to alter membrane electrostatics. This 'electrically active' transition is particularly intriguing, in part because the free energy difference between the conformers involved is expected to be potential-dependent [1,2].

The electrostatic changes that result from an electrically active transition can provide information on the nature of the transition [3,4]. In the native disk membrane, these electrostatic changes are consistent with an interfacial transfer of charge into a low-dielectric region of the membrane interior. Here we show that this conformational event can be reconstituted into model membrane systems containing purified rhodopsin and that the electrical changes produced are consistent with the previously proposed charge-transfer mechanism. In an attempt to examine the dependence of this transition on surface charge density, we also find a strong dependence of this charge-transfer event upon the phospholipid composition of the recombinant membrane.

The measurements described below were made in reconstituted membrane vesicles containing purified bovine rhodopsin. The rhodopsin was obtained from frozen, dark-adapted retinas (J. Lawson, Lincoln, NE) and purified by hydroxyapatite chromatography in dodecyltrimethylammonium bromide as previously described [5]. Egg phosphatidylcholine (PC) was obtained from fresh hen

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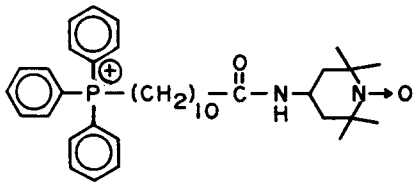
eggs and purified on alumina oxide [6]. Egg phosphatidylethanolamine (PE) was purified from a crude egg yolk extract [6] by salicic acid chromatography (Merck silica gel 60) in a chloroform/methanol gradient. Both egg PC and PE were stored in chloroform under an argon atmosphere at −20°C. Phosphatidic acid (PA) was obtained by the enzymatic hydrolysis of egg PC as previously described [7]. Brain phosphatidylserine (PS) was obtained from Sigma Chemical Company (St. Louis, MO). Rhodopsin-phospholipid dispersions were prepared as previously described [5,8]. Phosphate analysis was performed by the procedure of Bartlett et al. [9] and rhodopsin was assayed by absorption at 500 nm. Sonication of the dispersions was carried out using a probe type sonicator for brief periods (5 to 10 s) followed by an equivalent cooling period, to yield a total sonication time of approx. 2 min. The hydrophobic ion spin-label (I), shown below, phase partitions between membrane and aqueous phases. This partitioning depends upon the transmembrane potential, $\Delta\psi$, and two interfacial 'boundary' potentials (see McLaughlin, 1977, for a description of these potentials) [10]. This label was synthesized as previously described [2]. All EPR measurements were carried out on a modified Varian V-4500 series spectrometer.



In the presence of vesicles containing purified rhodopsin, label I was used to measure a photovoltage produced by the photolysis of rhodopsin. The probe changes its phase partitioning in response to this photovoltage so that the ratio of aqueous-to-membrane associated label increases. This change in phase partitioning for reconstituted membrane systems is shown in Fig. 1. Here the high-field resonance of I is monitored as a function of time. Because of the broad bound line-shape for this resonance, the amplitude of this line is easily calibrated to provide an accurate representation of the concentration of aqueous probe. The dependence of the phase partitioning (λ) upon

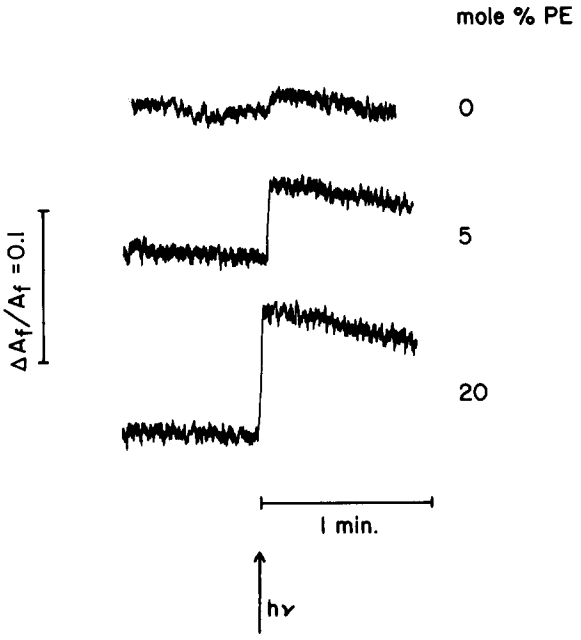


Fig. 1. A tracing of the high-field resonance of $2 \cdot 10^{-5}$ M phosphonium nitroxide I as a function of time in the presence of rhodopsin-containing vesicles. Rhodopsin is at a concentration of approx. 4 mg/ml and a ratio of protein : lipid of 1 : 100. The membrane sample was suspended in a low ionic strength medium of 5 mM Mes Buffer, pH = 5.5. The magnitude of the rhodopsin photovoltage is maximum at this pH [1]. As indicated, the three tracings were recorded from egg PC recombinant membranes that contained 0,5 and 20 mol% egg PE.

membrane voltages is given below in Equation 1.

$$\lambda = \frac{V_{m_i}/V_i\left(K_i e^{-\psi_i F/RT} + K_o V_{m_i} e^{(\Delta\psi - \psi_o)F/RT}\right)}{1 + V_o/V_i e^{\Delta\psi F/RT}} \quad (1)$$

Here K_i and K_o are binding constants of I to the internal and external surfaces of the vesicle and V_{m_i}, V_{m_o}, V_i and V_o are the volumes of the internal and external boundary regions and the internal and external aqueous volumes, respectively. These quantities are determined as previously described [2]. The voltages $\Delta\psi$, ψ_o and ψ_i refer to the transmembrane, the external boundary and internal boundary potential, respectively. Unlike native membrane systems, the photoresponses measured in the present reconstituted rhodopsin systems do not contain a transmembrane component; the photoresponse is not sensitive to the addition of ionophores such as valinomycin. As shown in Fig.

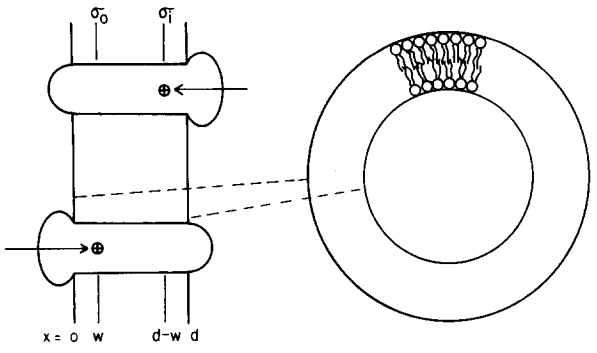


Fig. 2. A schematic drawing of the orientation of rhodopsin in this reconstituted membrane system. The boundary potential change is measured by the label I in the two interfacial regions at $x = w$ and $x = d - w$. Because both inside-out and right-side out orientations of rhodopsin occur in this membrane, no transmembrane potential component appears in the photovoltage detected by I.

2, an interfacial charge transfer should occur symmetrically in the reconstituted rhodopsin system, assuming no preferential orientation of rhodopsin. Thus, unlike the native system, the interfacial charge movement measured here is not expected to alter the potential between the two bulk aqueous phases, $\Delta\psi$ (note that if the vesicles had a sufficiently small radius of curvature and charge was moved a significant fraction of the distance through the bilayer, a transmembrane potential could result). The result seen here implies that the charge density change on each half of the bilayer must be approximately equal. The photoresponse measured here is identical to that seen in the native photoreceptor system in the presence of valinomycin.

To estimate the charge density transferred across the reconstituted membrane from λ, the phase partitioning of I, we first determine the dependence of λ upon the light-induced change in boundary potential. We will assume that the transmembrane potential is always near zero, and that the binding constants of the probe as well as the boundary potential and light-induced changes in boundary potential are equal on each side of the bilayer. The phase partitioning of the probe in the dark λ^d is given by Eqn. 1, above with $\Delta\psi$ set to zero. In the light, the phase partitioning λ^l is given by Eqn. 2, below.

$$\lambda^l = \frac{KV_{m_i}/V_i\left(e^{-\psi^l F/RT}\right)\left(1+V_{m_o}/V_{m_i}\right)}{\left(1+V_o/V_i\right)} \tag{2}$$

Here ψ^l is the light-induced change in the boundary potential on each interface. The binding constants and any free energy terms due to fixed charge density on the membrane interfaces are included in the binding constant K. As described previously, this light-induced phase partitioning can be determined from the partitioning of the probe in the dark and the fractional change in the free signal intensity of I as given in Eqn. 3.

$$\lambda^l = \left(\lambda^d - \Delta A_f/A_f\right)/\left(\Delta A_f/A_f + 1\right) \tag{3}$$

From Eqns. 1 and 2 it is easy to see that the light-induced change in boundary potential ψ^l is given by Eqn. 4.

$$\psi^l = (RT/F)\ln\left(\lambda^d/\lambda^l\right) \tag{4}$$

Thus, the phase partitioning of the hydrophobic ion probe I can be used to estimate the light-induced change in boundary potential. Note that the boundary potential, as defined here, is the sum of the surface potential and an interfacial potential drop between the membrane interface and the boundary region. To compare with previous measurements in native membrane systems, we have estimated the charge density transferred by rhodopsin in the reconstituted membrane system. We have used a simple model previously described [1] where charge is uniformly distributed in the plane of the membrane and the displacement of charge occurs through the boundary region *. In this case, the light-induced change in the boundary potential can be related to the charge density transferred (σ) and the dielectric constant of the boundary region (ϵ_b) by:

$$\psi^l = \psi_{so} + \frac{\omega}{\epsilon_b \epsilon_o}\sigma \tag{5}$$

Where ω is the displacement of the boundary region from the membrane interface and ψ_{so} is the

* This model is similar to the three-capacitor model employed for examining hydrophobic ion-induced boundary potentials [11]. With discrete charge on each rhodopsin, this model will likely underestimate the magnitude of the charge transfer [12]; nonetheless, it will give us an approximate estimation of the charge density transferred and will provide a comparison with previous measurements on native membrane systems.

surface potential contribution to the boundary potential change. ψ_{so} is readily determined from the charge density, σ, using the Gouy-Chapman theory as shown in Eqn. 6, where c is the concentration of monovalent salt and D is the dielectric constant of the aqueous medium.

$$\psi_{so} = (2RT/F)\sinh^{-1}(\sigma/c^{1/2})(500\pi/DRT) \quad (6)$$

Shown in Fig. 3 are values of the estimated charge densities for a series of rhodopsin recombinants in different mixtures of egg PC and egg PE. From the known densities of rhodopsin in these membranes, an estimate of the charge transferred per rhodopsin can be obtained. A comparison of the estimated charge densities can be made with native membrane systems from the known densities of rhodopsin in the two systems. In membranes containing 20% PE (Fig. 3), approx. 60% of the charge movement seen in the native system is measured. There was a large variability in the magnitude of the charge transfer between membrane preparations, as is apparent in Fig. 3. In reconstituted vesicles containing 20 mol% PE, many samples yielded values near the full native activity. A similar increase in the magnitude of the charge-transfer was observed upon the addition of phosphatidylserine (PS) or phosphatidic acid to egg PC containing vesicles.

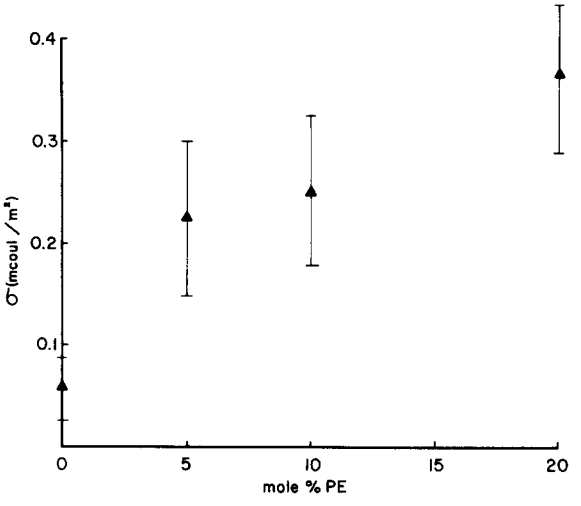


Fig. 3. A plot of the charge density transferred by rhodopsin as a function of the fraction of PE in the reconstituted membrane. The charge density is calculated according to Eqns. 5 and 6 with a total of three or four experiments for each point. The maximal charge density transferred to the recombinant membrane interface represents approx. 60% of that seen in the native membrane system. In the native membrane system, this change in charge density represents approx. 0.5 to 2 charges per rhodopsin. Error bars represent standard deviations for the data at each point.

The measurements that have been carried out here demonstrate that an electrically active conformational transition occurring in native rod outer segment disk membranes can be maintained when rhodopsin is reconstituted into certain model membrane systems. The measurement of this electrically active transition provides an additional assay of the 'native functionality' of rhodopsin in recombinant membrane systems. The orientation of rhodopsin in these reconstituted membrane systems has been shown to be symmetric [13] and the lack of a transmembrane potential component in the photovoltages produced by this system is further evidence of the interfacial transfer of charge proposed previously [1]. In native disk membranes the asymmetric movement of charge along a normal to the bilayer is apparently the source of a transmembrane photovoltage. As illustrated in Fig. 2, an equal density of rhodopsin on each vesicle surface results in an equivalent photovoltage at each interface, hence no net difference in potential between the two bulk aqueous phases is produced.

As shown above (Fig. 3), the photovoltage in recombinant systems containing egg PC is dramatically reduced compared to those containing additional egg PE. On average, σ was approx. 10% that seen in the native system. Within recombinants of a given composition, there was also a high degree of variability in the measured values of σ. The source of this variability is unclear. It did not appear to be related to the purity of rhodopsin or the oxidation state of its sulfhydryls. However, the detergent-purified rhodopsin used here may contain small, variable quantities of native phospholipid and variability due to this phospholipid seems likely. As seen from Eqn. 4, the size differences among vesicle preparations do not affect the measured photovoltages. Multilamellar structures could have affected measurements using I by not permitting this hydrophobic ion to reach equilibrium; however, the use of tetraphenylborate (to enhance the phosphonium migration rate [14]) as well as sonication of the rhodopsin-phospholi-

pid recombinants ensured that this was not a problem.

At present, the basis for this PE (PS or phosphatidic acid) requirement is unknown. An interfacial charge-transfer could be affected by the presence of surface charge and initial measurements were made with PS and phosphatidic acid additions to egg PC recombinants. While an increase in the photovoltage with negatively charged lipid might be expected (a more negative membrane surface should decrease the free energy of the photoactive conformer), the observation of similar enhancements with neutral lipid, PE, argues against the role of surface charge. Headgroup packing, hydrogen bonding or other physical changes in membrane structure are likely sources of the differences in rhodopsin function measured here.

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