letter to the editor

Surface charge density of purple membrane

Dear Sir:

Lipid analysis (Kates et al., 1982) and a variety of physical measurements (Fischer et al., 1978; Shinar et al., 1977; Keszthelyi, 1980; Neugebauer et al., 1978) indicate that purple membrane has a large negative surface change density σ. However, measurement of the magnitude of σ has produced conflicting results. Two groups have obtained -0.0005 charges/Å^2 (Ehrenberg and Berezin, 1984; Packer et al., 1984), whereas two other groups obtained values near -0.01 charges/Å^2 (Kuschmitz and Hess, 1981; Renthal and Cha, 1984). Ehrenberg and Berezin used a novel resonance Raman spectroscopy method. A cationic dye which undergoes concentration-dependent aggregation produces a new Raman line at 1,430 cm^{-1} that is proportional to the amount of aggregate. Purple membrane fragments were found to significantly increase the intensity of the 1,430 line, I_{1430}, and the effect was reversed by addition of KCl. The salt-dependence was used to estimate the surface change density via the Boltzmann relation and the Gouy equation. To do this calculation, the authors assume that the intensity of the 1,430 cm^{-1} line reflects the concentration of dye at the membrane surface. If the signal arises due to dye binding to the membrane surface, then it would in some way be proportional to the concentration of dye in solution at the surface. However, the Boltzmann and Gouy equations could not be applied without some information about the nature of the dye-membrane interaction (i.e., dissociation constant, number and kind of binding sites). If the signal arises entirely from dye aggregation (i.e., no binding), then the Poisson-Boltzmann equation may be applied to calculate the amount of aggregated dye in the double layer as a function of surface charge density, σ. This calculation requires several assumptions. The data presented by Ehrenberg and Berezin for free dye (Fig. 3 in their paper) suggests that the signal intensity at 1,446 cm^{-1}, I_{1446}, is independent of aggregate concentration, but rather reflects the total dye concentration. Furthermore, the signal at 1,430 cm^{-1}, I_{1430}, follows a dependence on dye concentration that is approximately consistent with dimer formation, with a dimer dissociation constant K_d of around 300 μM. Monomer and dimer may independently establish Boltzmann distributions of concentration, linked by the dimer dissociation constant.

$$K_d = \frac{(P_1)^2}{P_2} = \frac{[P_{1b}\exp(-\psi F/RT)]^2}{P_{2b}\exp(-2\psi F/RT)} = \frac{(P_{1b})^2\exp(-2\psi F/RT)}{P_{2b}\exp(-2\psi F/RT)} = \frac{(P_{1b})^2}{P_{2b}}, \quad (1)$$

where P_1 and P_2 are the concentrations at a distance x from the membrane, respectively, of monomer and dimer, and subscript b denotes the bulk solution concentration terms. The Raman intensity ratio reported by Ehrenberg and Berezin can be simplified if I_{1446} is dependent on total dye concentration.

$$\frac{P_i}{P_o} = \frac{(I_{1446}/I_{1430})_i}{(I_{1446}/I_{1430})_o} = \frac{(I_{1430})_o}{(I_{1430})_i} = \frac{P_{2b}}{P_{2d} + P_{2b}}, \quad (2)$$

where subscript o represents the term in the presence of very high salt and i at some lower salt concentration, and P_{2d} represents the total dimer in the double layer. P_{2d} may be obtained as follows.

Consider a patch of negatively charged membrane of area A, suspended in a medium containing a single type of monovalent cation-monovalent anion pair, each ion at a concentration C_b. The number of moles N of cation in the double layer out to a distance r from the surface is given by

$$N = A\int_0^r C\,dx, \quad (3)$$

where C is the concentration of the cation in a volume differential $A\,dx$ at a distance x from the membrane surface. Substitution for dx in terms of C may be obtained from

$$\frac{dC}{dx} = \frac{dC}{d\psi}\frac{d\psi}{dx}, \quad (4)$$

where ψ is the surface potential at a distance x from the membrane. Assuming a Boltzmann distribution of cations,

$$\frac{dC}{d\psi} = -FC/RT, \quad (5)$$

where F = Faraday's constant, R = the gas constant, and T = absolute temperature. The first integral of the Poisson-Boltzmann equation gives

$$\frac{d\psi}{dx} = \left[\frac{2\,RTC_b}{\epsilon\,\epsilon_0}(\exp(\psi\,F/RT) + \exp(-\psi F/RT) - 2)\right]^{1/2}, \quad (6)$$

where ϵ = the dielectric constant of water and ϵ_0 = vacuum permittivity. The Boltzmann relation provides a substitution for the exponential in Eq. 6 in terms of C and C_b.

$$\frac{d\psi}{dx} = \left[\frac{2\,RTC_b}{\epsilon\,\epsilon_0}\left(\frac{C_b}{C} + \frac{C}{C_b} - 2\right)\right]^{1/2}. \quad (7)$$

Substitution of Eqs. 4, 5, and 7 into Eq. 3 gives

$$N = \frac{AC_b^{1/2}}{K}\int_{C_m}^{C_r}\frac{C^{1/2}\,dC}{C_b - C}, \quad (8)$$

where $K = (2F^2C_b/\epsilon\,\epsilon_0 RT)^{1/2}$, and C_m and C_r are, respectively, the concentration of cation at the membrane surface, and the concentration of cation at a distance r from the membrane. K is

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the Debye reciprocal length for the membrane. Integration of Eq. 8 gives

$$N = \frac{A}{K}(C_b^{1/2})\,[2(C_m^{1/2} - C_r^{1/2})] - C_b^{1/2}\,ln\left[\frac{(C_r^{1/2} + C_b^{1/2})\,(C_m^{1/2} - C_b^{1/2})}{(C_r^{1/2} - C_b^{1/2})\,(C_m^{1/2} + C_b^{1/2})}\right]. \quad (9)$$

Application of Eq. 9 requires values for C_m, C_r, and C_b. Generally, only C_b is known. However, C_m and C_r may be calculated from Eq. 5 if the surface potential ψ is known as a function of distance. Eq. 6 shows the relationship between ψ and x (the integration of Eq. 6 is given by McLaughlin [1977]). One of the boundary conditions for Eq. 6 is the surface potential at the membrane surface, ψ_m. This may be obtained from the Gouy equation, which relates ψ_m to the surface charge density σ. Thus, the values of C_r and C_m may be calculated from C_b and σ. Typically C_b is set by the conditions of the experiment, and a wide variety of methods are available for measurement or modeling of σ (McLaughlin, 1977).

Eq. 9, in slightly modified form, also may be used to calculate the number of moles of a monovalent cation probe, P, that is present everywhere at concentrations far below C. Under these conditions, C_b determines the surface potential and the two terms in P_b vanish from the Poisson-Boltzmann equation. However, P must follow a Boltzmann distribution, and therefore the terms P_b/P and P/P_b may be substituted for the exponentials in Eq. 6. The result in Eq. 9 is to substitute P_b, P_r, and P_m for C_b, C_r, and C_m, respectively, in all terms except K. The choice of r in Eq. 9 is arbitrary, but in practice care must be exercised to avoid loss of significant figures in the $C_r^{1/2} - C_b^{1/2}$ term when $C_r \sim C_b$.

The number of moles of dimer in the double layer may be obtained by substituting $P_1 = (K_d P_2)^{1/2}$ for C in Eq. 8. P_{2d} is then calculated from the known concentration of bacteriorhodopsin and the average dimensions of the membrane sheets. Ehrenberg and Berezin's Raman data (P_i/P_o) as a function of salt concentration are replotted in Fig. 1. The calculated line assumes a surface change density of -0.5 charges/A^2. Thus, an unrealistically large surface charge is necessary to fit the data. The result suggests that the simple assumptions made here and in the paper by Ehrenberg and Berezin (that aggregation in the double layer gives rise to the enhanced 1,430 cm^{-1} signal in the presence of purple membrane) cannot be correct. A likely explanation is that some of the 1,430 cm^{-1} signal arises from the double layer and some from dye binding to the membrane. Regardless, it is not possible to extract a meaningful value for surface charge density from the data reported without additional information.

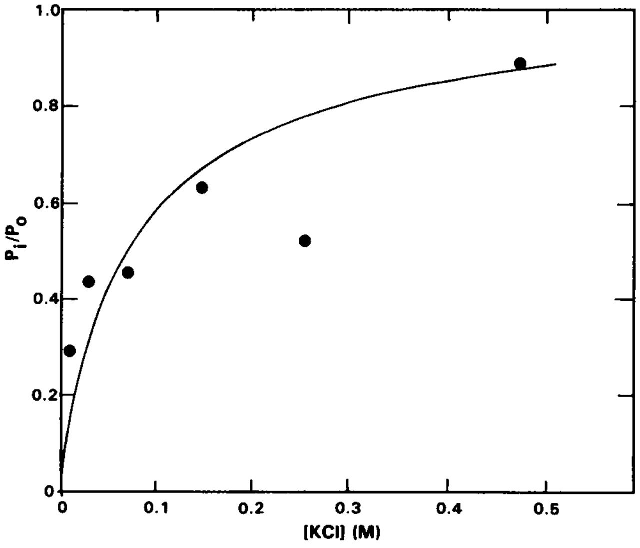

FIGURE 1 Aggregation of cationic dye as a function of purple membrane surface potential, varied by changing the salt concentration. Data replotted from Ehrenberg and Berezin (1984). Line is calculated from Eqs. 2 and 9, assuming the Raman intensity at 1,430 cm^{-1} is proportional to dimer concentration, and the surface charge density is -0.5 charges/Å^2. Unrealistically large surface charge density needed to fit data suggests part of the 1,430 cm^{-1} signal may arise from surface binding of the dye.

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