Light-induced Changes in H\(^+\) Binding to the Purple Membrane

EFFECT OF pH, LIGHT, TEMPERATURE, AND IONIC STRENGTH*

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Robert Renthal

From the Division of Earth and Physical Sciences, University of Texas at San Antonio, San Antonio, Texas 78285 and Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78284

Under continuous illumination, isolated planar sheets of purple membrane from *Halobacterium halobium* acido a the surroundings at alkaline pH. This light-induced change in H\(^+\) binding to the purple membrane (\(\Delta H\)) was studied by differential titration under varying conditions of pH, temperature, ionic strength, salt composition, light intensity, and wavelength. A maximum acidification was found between pH 9 and 10, with \(\Delta H\) less at neutral or more alkaline pH, consistent with a previously proposed three-state model. The light intensity and wavelength dependence also support this model. The temperature dependence of \(\Delta H\), interpreted in terms of the three-state model, is anomalous. The apparent enthalpy of proton dissociation (\(\Delta H^0\)) is \(-6\) kcal/mol, a value of opposite sign to the expected \(\Delta H^0\) for a group of pK \(\approx 10\). The apparent activation energy (\(E_a\)) for proton uptake is \(14\) kcal/mol in 15 mm NaCl and 18 kcal/mol in 3 M KCl, 5 to 10 times too large for a diffusion-limited proton transfer reaction from water. However, both \(\Delta H^0\) and \(E_a\) are consistent with conformational changes linked to light-independent proton dissociation and pump-dependent proton uptake. An increase in ionic strength increases \(\Delta H\). This effect is shown to be quantitatively explained by a high negative electrostatic surface potential, which accumulates protons in a diffuse electrical double layer.

The purple membrane of *Halobacterium halobium* contains a light-dependent transmembrane proton pump which can drive the synthesis of ATP (for a comprehensive review, see Ref. 1). Both the crystal structure (2) and amino acid sequence (3) of the light-absorbing protein bacteriorhodopsin have been determined, providing the basis for understanding a proton pump at the molecular level. When nonvesicular, planar fragments of the purple membrane are illuminated with a steady light-source, small pH changes may be measured in the surrounding medium, due to light-induced changes in proton binding to the purple membrane (\(\Delta H\)). This effect was first measured by Oesterhelt and Hess (4), in ether-saturated membrane suspensions at high ionic strength. I subsequently showed (5) that, at alkaline pH, \(\Delta H\) appears to depend on a group with a pK of 10. I also found \(\Delta H\) to be a sensitive probe of alterations in proton pump activity resulting from chemical modification of the purple membrane (6, 7). Garty et al. (8) have used the light-induced changes in proton binding as a measure of the relative rates of proton release and uptake.

Light-induced changes in proton binding may arise from a steady state build-up of protonated or deprotonated intermediates during proton pumping. Two types of proton binding sites could contribute to this effect: (I) amino acid side chains that directly participate in the proton translocation process, and (II) side chains that are perturbed during pumping (for example, by conformational changes). There could be some sites that combine both properties I and II, while others might be distinctly type I or type II.

Measurement of \(\Delta H\) provides unique information about side chains that undergo changes in protonation during proton pumping. The experiments reported here were undertaken in order do: 1) test the previously proposed three-state model for \(\Delta H\) (5), as a function of light intensity and wavelength; 2) measure the temperature dependence of \(\Delta H\); and 3) study the salt concentration dependence of \(\Delta H\). The temperature-dependence studies were initiated in the hope of identifying the group of pK 10 that participates in \(\Delta H\). The enthalpy of proton dissociation could distinguish between lysine and tyrosine, since \(\Delta H^0 = 6\) kcal/mol for phenolic groups, while \(\Delta H^0 > 10\) kcal/mol for amino groups. The salt concentration dependence was studied in the hope of clarifying the unusual finding of a salt-dependent stoichiometry for proton pumping which was one proton/cycle at low ionic strength and two protons/cycle at high ionic strength (9–11).

**MATERIALS AND METHODS**

Purple membranes were prepared by the method of Oesterhelt and Stoeckenius (12) from *H. halobium* S9. Experiments were done essentially as previously described (5). Unless otherwise indicated, the following conditions were used. Samples were 2.0 ml with purple membranes suspended at a concentration of 1.0 \(\times\) 10^-6 M bacteriorhodopsin in a specially-made 1.4-cm diameter vessel that was thermostatted to 15 °C by a Haake FK circulating constant-temperature bath. The incident light source was a quartz halogen movie projector lamp (Sylvania ELE) operated at 20 V, filtered through 7 cm of 1% CuSO\(_4\) and an Oriol 560 nm narrow band interference filter to an incident intensity of 2.4 \(\times\) 10^6 erg cm^-2 s^-1. Either a Beckman 39031 or Radiometer GK2321C electrode was used. The electrodes were wrapped in aluminum foil to within about 1 cm of the tip to prevent a light response. The pH was measured with either a Beckman 4500 or a Radiometer PHM 64 meter. The meter output was offset and amplified by a Gould 13-4615-10 differential amplifier, and recorded on either a Houston Instruments Omniscribe or Heath recorder at a scale of about 10^4 pH/inch. The light-induced change in proton binding to the membrane, \(\Delta h\), was calculated from the measured pH change, \(\Delta p\), and the measured buffering capacity (per mol of bacteriorhodopsin) of the membrane sample, \(B\):

\[\Delta h = B \Delta p\]

The buffering capacity was determined from the slope of the titration curve that was measured for each sample at the same time and under...
steady state light-induced change in $H^+$ binding to the purple membrane reaches a maximum value and then, above pH 10, is not observed at alkaline pH up to at least 10.5, and aggregation forms large aggregates. However, absorbance changes are observed at alkaline pH, purple membrane sheets acidify the medium under low intensity steady illumination, while at acid pH there is a net alkalization. This effect is more readily quantitated at alkaline pH than acid for two reasons: 1) the changes are larger per mol of bacteriorhodopsin, and 2) the membrane sheets are more constant in their physical properties above pH 7 than below. Near pH 3, the purple membrane turns blue and forms large aggregates. However, absorbance changes are not observed at alkaline pH up to at least 10.5, and aggregation above pH 7 only occurs at high ionic strength.

With decreasing $H^+$ concentration, the magnitude of the steady state light-induced change in $H^+$ binding to the purple membrane/mol of bacteriorhodopsin ($\Delta \delta$) increases. It reaches a maximum value and then, above pH 10, sharply decreases. The decrease is most obvious at lower temperatures (Figs. 1 and 2). The resulting curve is a second degree equation with the form

$$\Delta \delta = \frac{[H^+]}{A[H^+] + B[H^+] + C}$$

where $[H^+]$ is the hydrogen ion concentration and $A$, $B$, and $C$ are constants.

The physical significance of Equation 1 is most apparent when considering the magnitude of $\Delta \delta$ under conditions where the proton release and uptake kinetic constants have been independently measured by other methods. In deionized water, $\Delta \delta$ is too small to be accounted for only by light-induced deprotonation of photointermediates in the steady state. Such a two-state model would predict the following expression for $\Delta \delta$:

$$\Delta \delta = \frac{K}{K + [H^+]}$$

where $K$ is the ratio of the forward and reverse rate constants for light-induced deprotonation (4). For example, at pH 10, where the measured light-induced change in proton binding in deionized water is approximately 0.025 eq/mol (5), Equation 2 predicts a value of 0.14 eq/mol. Moreover, Equation 2 also predicts a steep rise to a limit of $\Delta \delta = 1$ at very alkaline pH, even at low light intensity. The measured values of $\Delta \delta$ are far below this predicted amount.

The $H^+$ binding sites that dissociate in the light must, at some alkaline pH, also dissociate in the dark. Thus, a more complete model for the light-induced change in proton binding is (5):

$$\Delta \delta = \left( \frac{[H^+]}{K_1 + [H^+]} \right) \left( \frac{K_2}{K_1 + [H^+]} \right)$$

where RH and R are, respectively, protonated and deprotonated bacteriorhodopsin that are not photochemically activated, and $R^*$ is deprotonated bacteriorhodopsin that is photocycling. The rate constant for light-induced proton release has been measured by flash spectroscopy (11, 14). However, under steady illumination, this rate is simply $Q_{Ia}$, where $Q$ is the quantum yield for proton pumping and $L$ is the absorbed light intensity. At the relatively low light intensities of the experiments reported here, only about one photon is absorbed/bacteriorhodopsin/s. The rate constant, $k_r$, refers to the reprotonation of bacteriorhodopsin during the protoII pump cycle. I assume that this proton uptake reaction occurs at the opposite side of the membrane from the light-induced proton release. Although this process can occur in the dark (i.e. for a few seconds after the light is turned off), the rate is dependent on the concentration of the photoproduct $R^*$. Measurements of $k_r$ by flash spectroscopy indicate that the rate is diffusion-limited (11, 14). Thus, at the light intensities used in the present experiments, appreciable steady state concentrations of $R^*$ will be found only at very low hydrogen ion concentrations (i.e. where $[H^+]k_1 = 1$). For convenience, $K_2$ is defined as $Q_{Ia}/k_r$, and is essentially constant, except as discussed below. $K_1$ is the dissociation constant for the photolabile $H^+$-binding when measured by titration in the dark. I assume that both the dissociation and association steps of the $K_1$ equilibrium are from the same side of the membrane (i.e. this equilibrium is not a transmembrane process, unlike the light-dependent proton release and uptake). Throughout this paper, I refer to the $K_1$ equilibrium as light-dependent proton dissociation, the $Q_{Ia}$ reaction as light-induced proton release, and the $k_r$ reaction as pump-dependent proton uptake.

Equation 3 predicts the correct second degree curve for $\Delta \delta$ versus $[H^+]$, as well as the correct magnitude for $\Delta \delta$. In Figs. 1 and 2, the peaks between pH 9 and 10 and the large decrease in $\Delta \delta$ to the alkaline side of the peak show that partial light-independent proton dissociation can well explain the observed dependence of $\Delta \delta$ on $[H^+]$. At pH values much lower than the peak value of $\Delta \delta$, it can be seen that the simple two-state model of Equation 2 and the three-state model of Equation 3 converge. Thus, at $[H^+]$ sufficient to protonate the...
photolabile binding site in the dark, predictions from the three-state model are similar to those from the two-state model.

Temperature Dependence of the Light-induced pH Changes—Figs. 1 and 2 show the effect of temperature on the \( \Delta \theta \) versus pH curves for two different ionic strengths. With decreasing temperature, \( \Delta \theta \) increases. This increase may be explained by assuming a temperature dependence of \( K_1 \) and \( k_1 \) in Equation 3. The data in Figs. 1 and 2 and also data at 10 and 20 °C (not shown), were fitted with lines using Equation 3. The constants \( K_1 \) and \( k_1 \) were obtained by an iterative computer method.

The temperature dependence of \( K_1 \) and \( k_1 \) are shown in Figs. 3 and 4 as van't Hoff and Arrhenius plots. From these plots, the apparent enthalpy of ionization (\( \Delta H^0 \)) and apparent activation energy (\( E_a \)) were calculated. For \( K_1 \), \( \Delta H^0 = -6 \) kcal/mol in 0.015 M NaCl and -6.5 kcal/mol in 3.0 M KCl. For \( k_1 \), \( E_a = 14 \) kcal/mol in 0.015 M NaCl and 18 kcal/mol in 3.0 M KCl. The data is summarized in Table I.

The temperature dependence of \( K_1 \) as previously noted, is approximately \( 10^{-37} \) m, near the ionization constant expected for a lysine or tyrosine side chain. However, \( \Delta H^0 \) clearly is of opposite sign to that expected for dissociation of a proton from lysine or tyrosine. The value of \( k_1 \) is about \( 10^{10} \) s \(^{-1}\), a rate constant for a diffusion-limited reaction.

The activation energy for the diffusion-limited proton transfer reaction \( H^+ + ^{16}O^2H_2O \rightarrow H_2O + ^{16}OH \) is 2 or 3 kcal/mol (15). But the apparent \( E_a \) for \( k_1 \) is 5 to 10 times too large for a diffusion-limited proton transfer reaction from water.

Variation of Incident Light Intensity—The magnitude of \( \Delta \theta \) may also be increased by increasing the incident light intensity. Equation 3 predicts a light-intensity dependence through \( K_2 \):

\[
K_2 = \frac{Q_{\text{e}}}{[\text{RH}]} (1 - 10^{-\epsilon'(\text{RH})})
\]

where \( Q_{\text{e}} \) is the incident light intensity and \( \epsilon' \) is the molar extinction coefficient. The factor on the right-hand side of Equation 3 predicts a linear relationship between 1/\( \Delta \theta \) and 1/\( Q_{\text{e}} \). Fig. 5 shows the intensity dependence of \( \Delta \theta \) plotted in double reciprocal form for purple membrane at 15 °C in 0.015 M NaCl, pH 9.0.

A straight line is found, with the measured slope of \( 5 \times 10^{-7} \) einstein/s in good agreement with a slope of \( 6 \times 10^{-7} \) einstein/s calculated from Equation 3. A linear double-reciprocal plot is also found at pH 8 (measured slope = \( 3 \times 10^{-7} \), calculated = \( 5 \times 10^{-6} \) einstein/s). As shown in Equation 4, \( K_2 \) is actually a function of bacteriorhodopsin concentration. Significant amounts of bacteriorhodopsin with the photosensitive H"-binding site unprotonated in the dark can exist at or above a pH comparable to pK. Thus, the value of [RH] in Equation 4 will decrease with increasing pH. However, as [RH] approaches 0, the term \( (1 - 10^{-\epsilon'(\text{RH})})/[\text{RH}] \) approaches 2.3 \( \epsilon' \) as an upper limit. Thus, even if all the RH were converted to R and R*, \( K_2 \) would increase by only a factor of 1.9. At pH 10, this results in only a small correction to calculated values of \( \Delta \theta \) (5). Thus, for most of the range from pH 7 to 10, the approximation of [RH] by total bacteriorhodopsin is a good approximation.

Variation of Wavelength—The wavelength dependence of \( \Delta \theta \) was studied with a series of narrow band interference filters. The measured values of \( \Delta \theta \) at pH 9.0 were corrected for the differences in transmittance between the filters, as recorded by a radiometer. The results are plotted superimposed on an absorbance spectrum of purple membrane in Fig. 6. The good agreement between the experimental points and
the purple membrane extinction indicates that absorbance by photoreaction cycle intermediates has little effect on $\Delta h$.

**Variation of Salt Concentration and Composition**—The effect of ions was tested by measuring $\Delta h$ near pH 9 in different concentrations of various salts. The values of $\Delta h$ shown in Fig. 7 were interpolated to pH 9.0 between approximately pH 8.5 and 9.5. The results show increasing steady state light-induced changes in proton binding with increasing ionic strength. The value of $\Delta h$ increases by a factor of four between 0.015 and 0.5 M NaCl. There is no significant difference between the effect of Na$^+$ and K$^+$, within the accuracy of the measurement technique. The presence of a divalent anion, SO$_4^{2-}$, also has little effect. However, divalent cations have a much larger effect on $\Delta h$ than monovalent cations. For example, a Ca$^{2+}$ concentration of 10 mM has a similar effect to 70 mM Na$^+$. Ca$^{2+}$ and Mg$^{2+}$ have approximately the same effect, except at high concentrations. (The effects of divalent ions could not be explored above about 30 mM, due to the extensive Ca$^{2+}$-induced aggregation of the membrane and the formation of Mg(OH)$_2$.) Thus, the results show an effect of salt concentration and cation charge on $\Delta h$.

Both the charge and concentration effects of salt can be explained by considering the presence of surface charge on the purple membrane. A negative surface charge will result in a surface potential, $\psi$, that attracts cations to the membrane surface in a diffuse double layer. The cation concentration near the membrane surface, $C_m$, is greater than the bulk solution concentration, $C_b$, by an exponential factor (see Refs. 16 and 17 for recent reviews):

$$C_m = C_b \exp(-\psi F/RT)$$

where $F$ = Faraday's constant, $R$ = the gas constant, and $T$ = absolute temperature. This increased surface cation concentration can affect both the measurement of ion release from, and uptake by, a transmembrane cation pump. The effect on release will be to diminish the concentration of released ions measured in bulk solution, since some ions that are pumped across the membrane could stay near the surface in the diffuse double layer. Ion uptake is affected by surface charge because it is a second-order process. The uptake rate will be enhanced by an increased surface concentration of the ion to be translocated.

Under the conditions of the experiments reported here, there is only a negligible effect on the rate of light-induced proton release, for the following reasons. The effect of surface charge on the light-induced proton release reaction will appear to be simply a buffering effect. The magnitude of $\Delta h$ in these experiments is quantitated by measuring the buffering capacity of the membrane. Thus, protons released into the diffuse double layer are largely taken into account. However, any light-induced changes in buffering would not be known, since the buffering capacity is measured in the dark. But this is also a negligible effect. Carmeli et al. (18) found about one negative charge appears on the purple membrane surface/bacteriorhodopsin during the formation of photointermediate M. At the bacteriorhodopsin concentrations and pH used in the experiments in Fig. 7, this light-induced change in surface charge would not attract a measurable amount of H$^+$ (5 x 10$^{-8}$ mol of H$^+$/mol of bacteriorhodopsin, or approximately 10$^{-8}$ of a pH unit, assuming all bacteriorhodopsins are pumping.)

The action of surface charge on proton uptake can readily account for the observed effects on light-induced changes in H$^+$ binding. The exponential factor in Equation 5 introduces a correction to the H$^+$ concentration $C_m$ which varies with the salt concentration, and consequently so will $\Delta h$. Although a variety of models can be used (16, 17), a simple...
discrete charge model was used to fit the data in Fig. 7.
\[ \psi(r) = \frac{n q}{4 \pi \varepsilon_0 \varepsilon} \exp(-\kappa r) \]  
(7)
where \( n \) is the number of charges, \( r \) is the distance from the proton uptake site, \( q \) is the unit charge, \( \varepsilon \) is the dielectric constant of water, \( \varepsilon_0 \) is the permittivity of free space, and
\[ \kappa = \left( \frac{F^2}{\varepsilon_0 R T} \sum c_i z_i^2 \right)^{1/2} \]  
(8)
where \( c_i \) is the concentration of \( i \)th ionic species and \( z_i \) is the charge of \( i \)th ionic species. The \( H^+ \) uptake site of the proton pump (which is assumed to be the same as the photosensitive \( H^+ \)-binding site) was placed at the center of a circle of \( r = 20 \) Å radius containing \( n = 15 \) negative point charges. This geometry and charge density is consistent with available structural (2) and compositional (3, 20) data. The constants \( K'_1 \) and \( K'_2 \) in Equation 6 are independent of salt concentration, unlike the analogous constants \( K_1 \) and \( K_2 \) in Equation 3. The values of \( K'_1 \) and \( K'_2 \) were calculated from the fact that \( K'_1 = K_1 Y' \) and \( K'_2 = K_2 Y' \). Using \( K_1 \) and \( k_1 \) from Table I, 15 mM NaCl, 15 °C, the dashed lines in Fig. 7 were obtained from Equations 6–8. The solid lines, which give a somewhat better fit, assumed \( K'_1 = 3 \times 10^{-10} \) M and \( K'_2 = 2 \times 10^{-10} \) M.

In order to fit the divergent ion data, it was necessary to assume that the divergent ions bind to negative sites, further reducing the surface charge. With divergent cation binding, Equation 7 becomes
\[ \psi(r) = \frac{n q}{4 \pi \varepsilon_0 \varepsilon R T (1 + C_i Y/K_i)} \exp(-\kappa r) \]  
(9)
where \( C_i \) is the bulk concentration of divergent ion and \( K_i \) is the intrinsic dissociation constant of the cation from a double negative site. The lines in Fig. 7 for the divergent cations were calculated assuming \( K_2 = 25 \) mM. Since \( Y \) in Equation 9 is a function of \( \psi \), the equation was solved by an iterative method. The calculated lines in Fig. 7 are presented as illustrations, since many of the parameters of Equations 5–9 have not been measured experimentally. The relatively poor fit of the calculated lines at high concentrations of divergent cations may be due to a slight effect of salt on \( K'_1 \). If, for example, calcium binds competitively to the dissociated state of the photosensitive \( H^+ \)-binding site (i.e. \( R \) in Equation 3), then \( K'_1 \) would increase and \( \Delta \kappa \) would decrease with increasing salt concentration.

**DISCUSSION**

**Steady-State Model**—The steady state model previously proposed (5) (Equation 3) appears to be the simplest that adequately describes the light-induced changes in \( H^+ \) binding to the purple membrane (\( \Delta \kappa \)). In accordance with Equation 3, the results presented here show that above \( pH 9 \), \( \Delta \kappa \) reaches a peak and then decreases (Figs. 1 and 2). The temperature dependence of \( \Delta \kappa \) may be explained by linear van’t Hoff and Arrhenius plots of the equilibrium and kinetic constants in Equation 3 (Figs. 1–4). The light intensity dependence is consistent with Equations 3 and 4 (Fig. 5). The wavelength dependence indicates that bacteriorhodopsin is the only significant absorbing species in the steady state at the actinic wavelength (Fig. 6). A membrane surface charge model (Equations 5–9) quantitatively accounts for the salt concentration dependence of \( \Delta \kappa \).

**Temperature Dependence**—The apparent enthalpy of ionization for \( K_1 \) (Equation 3 and Fig. 3) is of opposite sign to the expected value for amino acid side chains of \( pK \sim 10 \) (phenolic or amino groups). However, anomalous heats of proton dissociation are known to occur in enzyme-inhibitor complexes (21) or in proteins undergoing conformational changes (22, 23). The Arrhenius plot for the apparent rate of proton uptake (Fig. 4) gives an activation energy of 14–18 kcal/mol. This energy barrier is an order of magnitude too high for a diffusion-limited proton transfer reaction from water (15). Ort and Parson (24) reported a large activation energy for pump-dependent proton uptake, observed by measuring the temperature dependence of rates of light-induced volume changes (\( \Delta V \)) of purple membrane fragments. They found a nonlinear Arrhenius plot, with an apparent inflection point at 17 °C. The data presented in Fig. 4 appears to be linear, but it is not entirely comparable to the \( \Delta V \) rates. The apparent rate constant \( k_a \) in Fig. 4 is a second order rate constant, while that of Ref. 24 is first order. The rate of \( \Delta V \) was shown by Ort and Parson (25) to be pH-dependent during \( H^+ \) uptake suggesting a second-order component. The \( \Delta V \) rate also includes contributions from the buffer, while the rate constant \( k_a \) was measured in the absence of buffer. Despite these limitations of comparison, the apparent activation energy for proton uptake is a large conformational change in bacteriorhodopsin during pumping. Ort and Parson (24) find a large decrease in entropy during proton release and suggest this increase in order is due to a conformational change. Similarly, the large temperature dependence for pump-dependent proton uptake reflects the motions of bacteriorhodopsin toward its initial conformation. The slowing of this conformational motion at lower temperatures would thus slow proton uptake. Bacteriorhodopsin conformational changes have also been previously proposed to explain a variety of spectroscopic (26), kinetic (27), and reactivity (28) observations.

Can the conformational change and proton uptake processes be resolved into separate rates? Modifications of Equation 3 were derived with separate rate constants for conformational change (\( k_c \)) and proton uptake (\( k_p \)).

\[ \text{RH} \xrightleftharpoons[k_p]{k_c} \text{R}^+ \]

However, it was not possible to fit the temperature dependence of \( \Delta \kappa \) through the variation of \( k_c \) alone. Instead, equations for \( \Delta \kappa \) with the correct temperature dependence must have the temperature-dependent terms as products of [\( H^+ \)]. This strongly implies that the conformational change is directly linked to proton uptake. It is not yet clear whether the conformational change occurring upon light-independent proton dissociation is related to that occurring with pump-dependent proton uptake. In theory, bacteriorhodopsin need not participate in proton pumping other than to provide two wells leading to a Schiff base impeller at the center of the membrane. However, the evidence presented here strongly supports the notion of active participation of bacteriorhodopsin through conformational changes linked to proton transfer.

**Salt Concentration Dependence**—To resolve this statement, the Gouy-Chapman theory quantitatively accounts for the effect of salt concentration on the light-induced changes in \( H^+ \) binding. At low ionic strength, a significantly higher hydrogen ion concentration will be present in the diffuse double layer at the membrane surface than in bulk solution, while at high ionic strength, the surface and bulk hydrogen ion concentrations will be similar. The second order proton uptake reaction
of the proton pump is very sensitive to changes in the surface pH. In contrast, the effect of surface charge on the proton-release reaction is not apparent, since the measurement technique corrects for salt-induced changes in buffering capacity. Several workers have observed, under a variety of conditions and by a variety of methods, that the proton pump stoichiometry is apparently affected by salt concentration (9–11). Although I have assumed a value of one proton/cycle in order to calculate the kinetic constants given in Table I, the results presented here are not dependent on a particular pump stoichiometry. It is interesting that the surface charge model accounts for the salt concentration dependence of \( K_p \) without assuming any salt-induced changes in the quantum yield (contained in \( K_p \) of Equation 6; cf. Equation 4). It may be worthwhile to re-examine the methods used to measure the stoichiometry of proton pumping. The sensitivity of indicator dyes (11, 14) or buffers (9) could be influenced by surface charge at low ionic strength.

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