INTRINSIC ELECTRIC FIELDS AND PROTON DIFFUSION IN IMMOBILIZED PROTEIN MEMBRANES

EFFECTS OF ELECTROLYTES AND BUFFERS

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ABSTRACT We present a theory for proton diffusion through an immobilized protein membrane perfused with an electrolyte and a buffer. Using a Nernst-Planck equation for each species and assuming local charge neutrality, we obtain two coupled nonlinear diffusion equations with *new* diffusion coefficients dependent on the concentration of all species, the diffusion constants or mobilities of the buffers and salts, the pH-derivative of the titration curves of the mobile buffer and the immobilized protein, and the derivative with respect to ionic strength of the protein titration curve. Transient time scales are locally pH-dependent because of protonation-deprotonation reactions with the fixed protein and are ionic strength-dependent because salts provide charge carriers to shield internal electric fields. Intrinsic electric fields arise proportional to the gradient of an "effective" charge concentration. The field may reverse locally if buffer concentrations are large (≥ 0.1 M) and if the diffusivity of the electrolyte species is sufficiently small. The "ideal" electrolyte case (where each species has the same diffusivity) reduces to a simple form. We apply these theoretical considerations to membranes composed of papain and bovine serum albumin (BSA) and show that intrinsic electric fields greatly enhance the mobility of protons when the ionic strength of the salts is smaller than 0.1 M. These results are consistent with experiments where pH changes are observed to depend strongly on buffer, salt, and proton concentrations in baths adjacent to the membranes.

INTRODUCTION

In this paper we examine the diffusion of protons in synthetic immobilized protein and enzyme membranes perfused with a buffer and a salt. These protein membranes are composed largely of water (90% by weight) and are much thicker (15–100 μm) than synthetic lipid bilayer membranes (50–100 Å). Because the membranes are mainly composed of water, we assume the dielectric constant is the same inside and outside.

Bass and Moore (2) have reviewed the important role of pH on the conductivity of nerve membranes, which they indicated are 40% protein (p. 157). Mosbach (12) and Goldstein and Katchalski-Katzir (7) have reviewed recent progress in immobilized enzymes. They point out that few enzymes exist in vivo as free protein molecules in an aqueous environment, but are usually membrane bound (e.g., those found in the mito-

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chondria). Naparstek et al. (13) and Graves et al. (8) found unusual transient and oscillatory phenomena for reactions in immobilized enzyme systems. Hence, a theoretical and quantitative understanding of time-dependent processes in the microenvironment of fixed proteins is needed. In this paper we show how proton (H^+ ion) diffusion is modulated (facilitated or inhibited) in protein membranes at small-but-finite ionic strengths by self-consistent internal electric fields.

In a previous work Deem et al. (4) derived the nondimensional association-dissociation time scale factor $\tau(H)$, which multiplies the time derivative in a diffusion equation. As indicated, the relaxation time τ is pH-dependent because immobile proteins will rapidly and reversibly associate and dissociate H^+ ions in accordance with their titration curve $\bar{r}(H)$. This is an analogue of the reversible sorption and desorption of small molecules on solid supports, or immobilized polymers. (For a recent review and references, see Vieth et al. [17].) Also, it was shown that mobile buffers can provide carriers to facilitate the net transport of H^+ ions. However, intrinsic electric fields can play a role. Fig. 1 shows the pH measured by an electrode on which is cast a 4:1 papain: bovine serum albumin (BSA) membrane 15 μm thick (4). At $t = 0$, the membrane-coated electrode is transferred from a well-stirred bath at pH = 6.5 to another well-stirred bath at pH = 9.5. For baths with no salt, the time scale for diffusion is significantly smaller than for a 0.2 M KCl bath. Above this concentration (0.2–1.0 M) there is a very small change in the time scale.

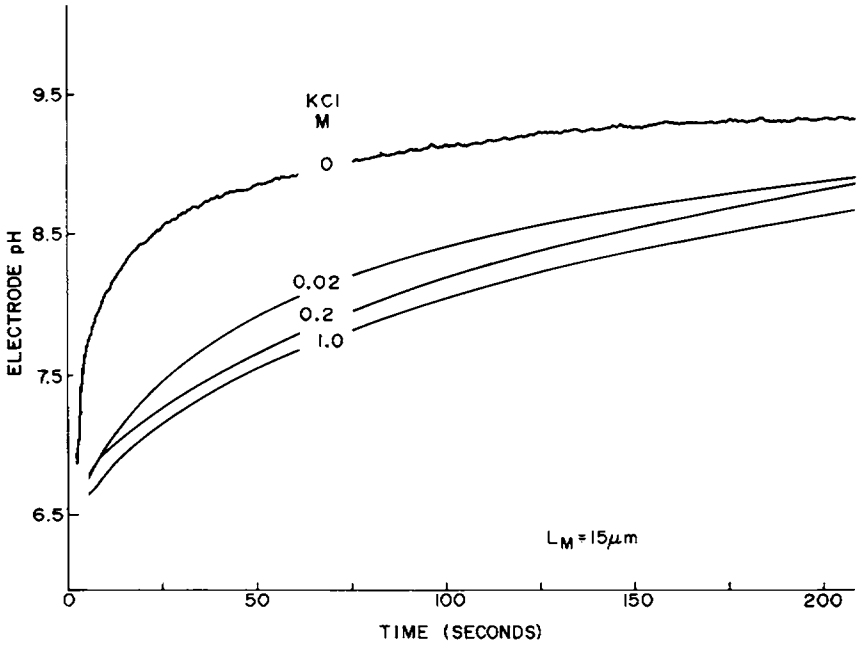

FIGURE 1 Experimental response times for a 15 μm papain/BSA membrane immobilized on a glass pH electrode with varying bath salt concentrations. The electrode, initially at pH 6.5, is immersed into the well-stirred bath fixed at pH 9.5. Both baths have the same salt concentration and no buffer.

In the present work we show how a single univalent electrolyte modifies the diffusion of H^+ ions in a protein membrane. Our formulation resembles "ambipolar" diffusion theory in electrolytes (6) or gaseous plasmas (11). The electric field is obtained from an asymptotic description consistent with microscopic charge neutrality where $\bar{\lambda}_D^2/L_M^2 \ll 1$; that is, "relaxation" times, proportional to $\bar{\lambda}_D^2/D_H$, are very small compared to diffusion times, proportional to L_M^2/D_H. Here $\bar{\lambda}_D$ is an "effective" Debye length, L_M is the membrane thickness, and D_H is the H^+ ion diffusivity, assumed to be constant. In essence, we find that if the ionic strength of salts is >0.1 M, they provide ions that "short out" the electric fields and one recovers $\tau(H)$ of the previous work.

GLOSSARY OF SYMBOLS[1]

$a_\pm = D_{s\pm}/D_H$	Normalized diffusivity of positively and negatively charged salt species (Eq. 9).
A_T	Total concentration of fixed protein (Eq. 9).
B_T	Total concentration of mobile buffer (Eq. 10).
C_k	Concentration of species k (mol/liter) (Eq. 1).
c_A, c_B	Number of cationic groups on protein (A) or buffer (B) (Eqs. 9, 10).
$C_{s\pm}$	Concentration of positively and negatively charged salt species (Eq. 15).
D_k	Diffusivity of species k: D_B (buffer); D_H (hydrogen-ion); D_{OH} (hydroxyl ion); D_s (salt ion) (Eq. 1).
D_e, D_{se}	Effective diffusion parameters in the ideal electrolyte limit ($\eta = 0$) (Eqs. 52 and 53) ($D_{11} \to D_e$, $D_{21} \to D_{se}$).
e	Electronic charge magnitude (Eq. 1).
$\mathbf{E}(x,t)$	Electric field vector (Eqs. 3, 4).
$E(x,t)$	x-component of E (Eq. 25).
$E^* = (L_M k_B T/e)E$	Normalized electric field (Eq. 27).
I	Ionic strength (after Eqs. 8 and 18).
k_B	Boltzmann's constant (Eq. 1).
K_W	Dissociation constant of water = 10^{-14} (mol/liter)^2 (Eq. 10).
K_n^B	Dissociation constants for intermediate buffer states (Eq. 12).
L_M	Membrane thickness (Eq. 27).
N_A	Avogadro's number (Eq. 5).
q	Electric charge associated with proton-related species, $q = q(H,I)$ (Eqs. 3, 9).
q_f	Free electric charge associated with proton-related species (Eq. 6).
q_b	Bound electric charge associated with proton-related species (Eq. 6).
q_s	Electric charge associated with salt-related species (Eqs. 4, 15).
Q	Effective concentration of hydrogen-ion related species (Eqs. 3, 8, 11).
Q_s	Effective concentration of salt-related species (Eqs. 4, 17).
$\bar{Q}$	Effective concentration normalizing constant (Eq. 26)
$\bar{r}_A, \bar{r}_B$	Titration curve of protein (A) and buffer (B) $\bar{r}_A = \bar{r}_A(H,I)$ and $\bar{r}_B = r_B(H)$ (Eqs. 9, 10, 11, and 14, and Fig. 3).
R_{kl}	Chemical reaction terms (Eq. 1).
$t_D = L_M^2/D_H$	Diffusion time constant (Eq. 31).
$t_R = \lambda_D^2/D_H$	Relaxation time parameter (Eq. 29).
$\bar{t}_R = \bar{\lambda}_D^2/D_H$	Relaxation time constant (Eq. 29).
z_k	Valence number of species k (Eq. 1).

[1] cgs units are used unless otherwise indicated.

∂_x, ∂_t Partial derivatives with respect to x and t (Eq. 1).
Δ Effective flux of hydrogen-ion related species $\Delta = \Delta(H)$ (Eqs. 3, 7, 10).
Δ_s Effective flux of salt-ion related species (Eqs. 4, 16, 20, 37*a*).
ϵ_0 Dielectric constant (Eq. 5).
ϵ Small-perturbation expansion parameter (Eq. 31).
η Normalized electrolyte diffusivity difference (Eq. 22*a*).
λ_D^2 Effective Debye length (after Eq. 29).
$\overline{\lambda}_D^2$ Normalizing constant effective Debye length (Eq. 28).
μ_k Mobility of species k (Eq. 1).
ξ Normalized electrolyte diffusivity average (Eq. 22*b*).
$\tau = \tau(H)$ Association-dissociation time-scale factor $= (\partial q/\partial H)/(\partial \Delta/\partial H)$ (Eq. 49).

THEORY

We begin with a Nernst-Planck description for each mobile species with no external electric field imposed. We obtain a coupled set of nonlinear diffusion equations and an equation for the *intrinsic* electric field dependent on charge concentrations and their gradients. We assume that the titration curve of the immobilized protein, $\bar{r}_A(H)$, is not affected by the immobilization procedure. We treat the case of thick membranes that are mainly water; that is, we neglect image-potentials that seem to play a prominent role in thin lipid bilayer membranes (1, 9). In this paper we do not examine boundary conditions at membrane-solution interfaces, which should include Donnan potentials and electrostatic partition coefficients.

In Fig. 2 we show a model of a one-dimensional membrane of thickness L_M. It is bounded on one side by an impermeable plane (that could represent a glass pH electrode) and on the other side by a bath of controlled ionic concentrations. The membrane consists of immobilized proteins A, through which diffuses an ionic solution consisting of protons H^+, hydroxyl ions OH^-, buffer ions B, and salt ions s. We sep-

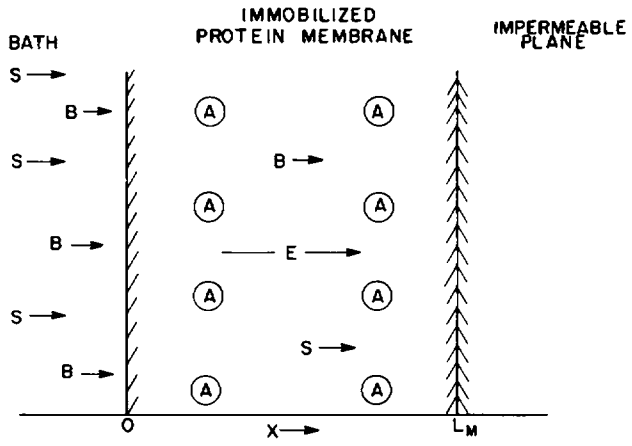

FIGURE 2 One-dimensional model of a porous membrane consisting of immobilized proteins A. The membrane is bounded on the right by an impermeable plane and on the left by an aqueous solution of a univalent electrolyte s and an ideal buffer B.

arate the charged species into two parts: q from H^+ ions and species which rapidly and reversibly associate and dissociate with H^+ ions, such as hydroxyls, proteins, and pH buffers; and q_s from salt species, which we assume are always dissociated and do not interact chemically with protons or with the fixed protein. The units of q and q_s are moles per liter (number density) and they can be converted to charge density by multiplication by Faraday's constant. We shall see that q and q_s are coupled only through the intrinsic electric field, omitted from our previous investigation (4).

The individual Nernst-Planck species evolution equations are

$$\partial_t C_k = \nabla \cdot [D_k \nabla C_k - z_k \mu_k C_k \mathbf{E}] + \sum_l R_{kl}, \tag{1}$$

where the species concentrations C_k (moles per liter) have valences z_k; molecular diffusivities D_k, mobilities μ_k, and R_{kl} represent terms due to chemical reactions between species k and l. For simplicity, we assume that mobilities and diffusivities are constants and are related by the Nernst-Einstein relation

$$D_k/\mu_k = k_B T/e, \tag{2}$$

where k_B is Boltzmann's constant, T is the absolute temperature, and e is the electronic charge. Boundary conditions consistent with Fig. 2 are $C_k(-\infty, t)$ = constant, and $E(-\infty, t) = E(L_M, t) = 0$. Also, if we impose a zero-flux boundary condition at $x = L_M$ for each species, then

$$\partial_x C_k(x,t)\Big|_{x=L_M} = 0.$$

The Nernst-Planck equations for q and q_s are obtained by multiplying Eqs. 1 by z_k and summing

$$\partial_t q = \nabla \cdot (D_H \nabla \Delta - \mu_H Q \mathbf{E}), \quad \text{(proton-related)}, \tag{3}$$

$$\partial_t q_s = \nabla \cdot (D_H \nabla \Delta_s - \mu_H Q_s \mathbf{E}), \quad \text{(salts)}, \tag{4}$$

where the reaction terms cancel identically due to conservation of charge. D_H and μ_H are the constant molecular diffusivity and mobility of protons. We have introduced the macroscopic variables q, q_s, Q, Q_s, Δ, and Δ_s to simplify the analysis given below when the pseudo-steady-state hypothesis is introduced to treat the multitude of protein-proton-(OH) interactions.

Poisson's equation for the electric field is

$$\nabla \cdot \mathbf{E} = (4\pi e N_A/\epsilon_0)(q + q_s) = -\nabla^2 \phi. \tag{5}$$

Here ϵ_0 is the dielectric constant of the membrane-plus-solution system, ϕ is the electric potential, and N_A is Avogadro's number divided by 10^3 (or 6.02×10^{20}), so that E and ϕ are expressed in electrostatic units. q is the sum

$$q = q_f + q_b = \sum_{\text{nonsalts}} z_k C_k, \tag{6}$$

where the nonsalt free charges, q_f, include H^+, $(OH)^-$, and partially dissociated buffer species, and the bound charges, q_b, include partially dissociated immobilized protein species. q_s is defined as a sum over the free salt species. The effective charge function Δ is defined, as previously (4), to be a weighted sum over nonsalt free species

$$\Delta = \sum_{\substack{\text{free} \\ \text{nonsalts}}} z_k \frac{D_k}{D_H} C_k, \tag{7}$$

with a similar expression Δ_s for salts. The concentration function Q in Eq. 3 is defined by

$$Q = \sum_{\substack{\text{free} \\ \text{nonsalts}}} z_k^2 \frac{D_k}{D_H} C_k, \tag{8}$$

where we have used Eq. 2. A similar expression, Q_s, holds for salts. The definition of Q_s is similar to that of ionic strength of free salts, $I = \frac{1}{2} \Sigma z_k^2 C_k$, but is modified by weighting factors, namely mobility ratios. Thus, applying the pseudo-steady-state hypothesis (4), Eqs. 6, 7, and 8 become

$$q = H - K_W/H + B_T[c_B - \bar{r}_B(H)] + A_T[c_A - \bar{r}_A(H, I)], \tag{9}$$

$$\Delta = H - \frac{D_{OH}}{D_H} \frac{K_W}{H} + \frac{D_B}{D_H} B_T[c_B - \bar{r}_B(H)], \tag{10}$$

and

$$Q = H + \frac{D_{OH}}{D_H} \frac{K_W}{H} + \frac{D_B}{D_H} B_T[c_B^2 - 2c_B\bar{r}_B(H) + \overline{r_B^2}(H)]. \tag{11}$$

Here $(OH) = K_W/H$, D_{OH} is the molecular diffusion coefficient for hydroxyl ions, B_T is the total molar concentration of the mobile buffer in all partially dissociated forms, A_T is the total molar concentration of immobile protein, $\bar{r}_B$ and $\bar{r}_A$ are the titration curves of the buffer and fixed protein (as discussed below) and $K_W = 10^{-14}$ $(\text{mol/liter})^2$ is the dissociation constant for water. Note, at this stage we make the reasonable and very convenient assumption that the dissociation constants of the low molecular weight buffer, K_n^B, are independent of ionic strength (therefore, $\partial\bar{r}_B/\partial I = 0$), but introduce no such restriction on the protein dissociation constants K_n^A (therefore, $\partial\bar{r}_A/\partial I \neq 0$).

For convenience, we have assumed an "ideal" buffer; that is *all* partially dissociated buffer species have identical diffusion coefficients $[(D_k)_{\text{buffer}} = D_B]$, which factor out of buffer terms in Eqs. 7 and 8. Furthermore, the buffer is characterized by N_B rapid association-dissociation reactions having dissociation constants K_n^B. That is,

$$({}^{n-1}B) \underset{}{\overset{K_n^B}{\rightleftharpoons}} ({}^nB) + H^+, \quad (n = 1, \ldots N_B), \tag{12}$$

where $({}^nB)$ is the sum over all dissociated buffer species for which n protons have been removed. c_B is the number of buffer cationic groups ($c_B = 0$ for commonly used weak acids like H_3PO_4). $r_B(H)$ is the titration curve, often approximated by

$$\bar{r}_B(H) = \frac{\sum_{n=1}^{N_B} n \frac{K_1^B}{H} \cdots \frac{K_n^B}{H}}{1 + \sum_{n=1}^{N_B} \frac{K_1^B}{H} \cdots \frac{K_n^B}{H}} = -\frac{H}{\sum_0} \frac{\partial \sum_0}{\partial H} = \frac{\partial \log_{10} \sum_0}{\partial pH}, \tag{13}$$

and

$$\overline{r_B^2}(H) = \frac{\sum_{n=1}^{N_B} n^2 \frac{K_1^B}{H} \cdots \frac{K_n^B}{H}}{1 + \sum_{n=1}^{N_B} \frac{K_1^B}{H} \cdots \frac{K_N^B}{H}} = \frac{H}{\sum_0} \frac{\partial}{\partial H} \left(\frac{H \partial \sum_0}{\partial H} \right), \tag{14}$$

where $\sum_0$ is the denominator of Eq. 13:

$$\sum_0 = 1 + \sum_{n=1}^{N_B} \frac{K_1^B}{H} \cdots \frac{K_n^B}{H}.$$

A similar discussion applies for the protein, which we assume is characterized by N_A rapid association-dissociation reactions. However, we allow K_n^A to vary with I and thus $\bar{r}_A = \bar{r}_A(H, I)$. (For further details refer to Edsall and Wyman [5], pp. 487–492, and to Tanford [16], p. 570. Note that the former use $h(H)$ for the titration curve, while Tanford uses $\bar{r}(H)$.) Fig. 3 gives $\bar{r}_B(H)$ and $\overline{r_B^2}(H)$ for a phosphate buffer ($N_B = 3$, $c_B = 0$, $pK_1^B = 2.12$, $pK_2^B = 7.21$ and $pK_3^B = 12.3$).

The equations corresponding to Eqs. 9, 10, and 11 for a univalent electrolyte are

$$q_s = C_{s+} - C_{s-}, \tag{15}$$

$$\Delta_s = [a_+ C_{s+} - a_- C_{s-}], \tag{16}$$

$$Q_s = [a_+ C_{s+} + a_- C_{s-}], \tag{17}$$

and the ionic strength I is defined as

$$I = \tfrac{1}{2}(C_{s+} + C_{s-}) \tag{18}$$

where C_{s+} and C_{s-} are the concentrations of positive and negative salt species and

$$a_\pm = D_{s\pm}/D_H. \tag{19}$$

Solving Eqs. 15 and 18 for C_{s+} and C_{s-}, we can write Eqs. 16 and 17 as

$$\Delta_s = 2\eta I + \xi q_s, \tag{20}$$

and

$$Q_s = 2\xi I + \eta q_s, \tag{21}$$

where

$$\eta = \tfrac{1}{2}(a_+ - a_-), \tag{22a}$$

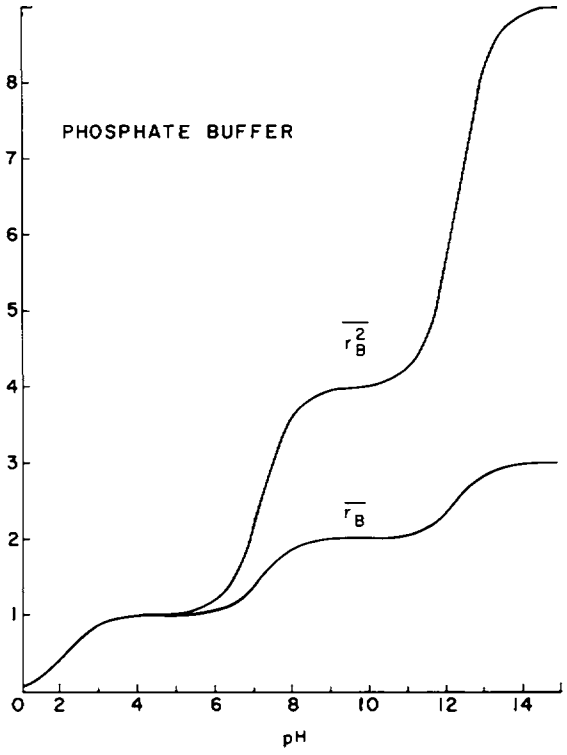

FIGURE 3

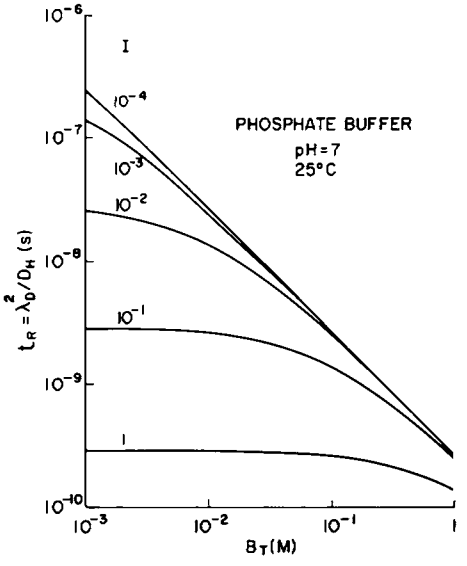

FIGURE 4

FIGURE 3 $\overline{r_B}(H)$ and $\overline{r_B^2}(H)$ vs. pH for a phosphate buffer $N_B = 3$, $c_B = 0$, $pK_1^B = 2.12$, $pk_2^B = 7.21$, and $pK_3^B = 12.3$.

FIGURE 4 Relaxation time t_R vs. total buffer concentration $B_T(M)$ at pH = 7.0 and various ionic strengths, I (M), (Eq. 29). An ideal phosphate buffer is assumed with $pK_1^B = 2.12$, $pK_2^B = 7.21$ and $pK_3^B = 12.3$ and $D_B = D_s = D_{OH} = 3.2 \times 10^{-6}$ (cm^2/s). $D_H = 5.5 \times 10^{-6}$ (cm^2/s). The dielectric constant is $\epsilon_0 = 78.5$ and $T = 25$°C.

and

$$\xi = \tfrac{1}{2}(a_+ + a_-). \tag{22b}$$

The "ideal" univalent electrolyte is one where we assume

$$a_+ = a_- = a \quad \text{or} \quad \eta = 0. \tag{23}$$

RAPID-RELAXATION APPROXIMATION FOR THE ELECTRIC FIELD

For membranes of biological dimensions or greater, we show that the electric field E responds rapidly to changes in the concentration gradients of mobile species. On a longer time scale, E varies in a quasi-steady-state manner with concentration gradients.

We assume the one-dimensional configuration shown in Fig. 2 and differentiate Eq. 5 with respect to t:

$$\partial_x E = (4\pi e N_A/\epsilon_0)\partial_t(q + q_s)$$
$$= (4\pi e N_A/\epsilon_0) D_H \partial_x\{\partial_x(\Delta + \Delta_s) - (k_B T/e)^{-1}(Q + Q_s)E\}, \quad (25)$$

where we have used Eqs. 3 and 4. We integrate Eq. 25 with respect to x and normalize E and obtain

$$\partial_t E^* = (D_H/\bar{\lambda}_D^2)[L_M \bar{Q}^{-1}\partial_x(\Delta + \Delta_s) - \bar{Q}^{-1}(Q + Q_s)E^*], \quad (26)$$

where

$$E^* = [L_M(k_B T/e)^{-1}]E, \quad (27)$$

$$\bar{\lambda}_D^2 = \epsilon_0 k_B T/4\pi e^2 N_A \bar{Q}, \quad (28)$$

and $\bar{Q}$ is a normalizing constant. We have omitted a time-dependent function of integration because of the boundary condition $E(L_M, t) = 0$ imposed previously.

The effective relaxation times

$$t_R = \lambda_D^2/D_H, \quad \text{and} \quad \bar{t}_R = \bar{\lambda}_D^2/D_H, \quad (29)$$

are very small. Here λ_D, an effective Debye length, is defined by $\lambda_D^2 = \bar{\lambda}_D^2[\bar{Q}/(Q + Q_s)]$. To obtain t_R, we have made the pseudo-steady-state approximation that all (fast) association-dissociation reactions involving protons and partially dissociated buffer and protein species (e.g., those represented by Eq. 12) are in equilibrium on the diffusion time scale, $t_D = L_M^2/D_H$. Fig. 4 gives computed values of t_R vs. B_T at pH = 7.0, using Eqs. 11 and 18 for Q and I, respectively. We assume an "ideal" phosphate buffer ($D_{kB} = D_B$); $D_H = 5.5 \times 10^{-6}$ cm^2/s, and $D_{OH} = 3.2 \times 10^{-6}$ (cm^2/s) (as given in reference 13) and for convenience $D_{OH} = D_B = D_s$; and $\epsilon_0 = 78.5$. The relaxation time t_R decreases with decreasing ϵ_0 and with increasing pH. Note that the knee of the curve occurs where $B_T \approx I$ and that the small-B_T asymptote is a function of pH and ionic strength I.

The extremely small relaxation times evident in Fig. 4 lead to the conclusion that the electric field will adjust rapidly and, on the diffusion time scale, t_D, will vary with the concentrations and their spatial gradients. That is, if we normalize t in Eq. 26 by $t_D (t^* = t/t_D)$, it becomes

$$\epsilon \partial_{t^*} E^* = [L_M \bar{Q}^{-1}\partial_x(\Delta + \Delta_s) - \bar{Q}^{-1}(Q + Q_s)E^*], \quad (30)$$

where

$$\epsilon = \bar{t}_R/t_D = \bar{\lambda}_D^2/L_M^2 \approx 2 \times 10^{-8}. \quad (31)$$

The small parameter ϵ was known to early investigators (14, 15, 3). MacGillivray (10) used ϵ as the basis for an asymptotic treatment of charge neutrality and Donnan potentials in a fixed-charge membrane with two diffusing species described by the Nernst-Planck-Poisson equations.

We now expand all dependent variables, namely E^*, $\bar{Q}^{-1}(\Delta + \Delta_s)$ and $\bar{Q}^{-1}(Q + Q_s)$, in the small parameter ϵ. For example, if $E^* = E^{*(0)} + \epsilon E^{*(1)} + \epsilon^2 E^{*(2)} \ldots$, then to lowest order:

$$E^{(0)} = (k_B T/e)(Q^{(0)} + Q_s^{(0)})^{-1}\partial_x(\Delta^{(0)} + \Delta_s^{(0)}), \quad (32)$$

and then to first order

$$E^{(1)} = \left(\frac{k_B T}{e}\right)\left\{\frac{\partial_x(\Delta^{(1)} + \Delta_s^{(1)})}{(Q^{(0)} + Q_s^{(0)})} - \frac{(Q^{(1)} + Q_s^{(1)})}{(Q^{(0)} + Q_s^{(0)})^2}\,\partial_x(\Delta^{(0)} + \Delta_s^{(0)}) - \bar{t}_R\,\bar{Q}(Q^{(0)} + Q_s^{(0)})^{-1}\partial_t[(Q^{(0)} + Q_s^{(0)})^{-1}\,\partial_x(\Delta^{(0)} + \Delta_s^{(0)})]\right\}, \quad (33)$$

where the zero- and first-order quantities of Δ, Δ_s, Q, and Q_s are obtained from their time evolution equations. In the following, we omit all first- and higher-order terms and suppress the superscript (0).

COUPLED NONLINEAR DIFFUSION EQUATIONS. (EFFECTIVE DIFFUSION COEFFICIENTS RESULTING FROM INTRINSIC ELECTRIC FIELDS)

The theoretical treatment given below resembles the theory of ambipolar diffusion in an electrolyte (6) or partially ionized gas (11). In the latter situation we have very mobile electrons trying to run away from positive ions but retarded by collisions with a nearly immobile background of neutrals. An electric field is generated proportional to $\nabla n (n \approx n_i \approx n_e)$ and the mean electron speed is reduced. The net diffusion constant is about twice the ion diffusion constant. Our analogy to the neutral background above is the immobilized protein that can facilitate or impede H^+ ion flow by protonation or deprotonation reactions. These processes generate an electric field, Eq. 32.

We will now obtain two coupled diffusion equations, one H^+ ion-related and one salt-related. We substitute Eq. 32 into the one-dimensional forms of Eqs. 3 and 4 and obtain the coupled nonlinear diffusion equations

$$\partial_t q = D_H \partial_x\left(\frac{Q_s}{Q + Q_s}\,\partial_x\Delta - \frac{Q}{Q + Q_s}\,\partial_x\Delta_s\right), \quad (34)$$

and

$$\partial_t q_s = D_H \partial_x\left(-\frac{Q_s}{Q + Q_s}\,\partial_x\Delta + \frac{Q}{Q + Q_s}\,\partial_x\Delta_s\right). \quad (35)$$

This is the zero-order approximation, in $\epsilon = (\lambda_D/L_M)^2 \ll 1$, and microscopic charge neutrality

$$q + q_s = 0 \quad (36)$$

is a consequence, as obtained by adding Eqs. 34 and 35. Thus Δ_s and Q_s, Eqs. 20 and 21, become

$$\Delta_s = 2\eta I - \xi q, \quad (37a)$$

$$Q_s = 2\xi I - \eta q. \quad (37b)$$

We adopt q and I as dependent variables. We now combine the primitive forms of the species equation (Eq. 1), or

$$\partial_t(C_{s+} + C_{s-}) = 2\partial_t I = D_H \partial_x\{\partial_x Q_s - E\Delta_s/k_B T e^{-1}\}, \quad (38)$$

where we have used Eq. 18 and $q_s = -q$. We then substitute Eq. 32 for E (with superscripts suppressed) and obtain

$$2\partial_t I = D_H \partial_x \{\partial_x Q_s - (Q + Q_s)^{-1} \Delta_s \partial_x (\Delta + \Delta_s)\}. \tag{39}$$

We eliminate derivatives of Δ_s and Q_s using Eqs. 37*a* and *b* and $\partial_x \Delta$ is obtained from

$$\partial_x \Delta = (\Delta_H)\partial_x H = \Delta_H [q_H^{-1} \partial_x q - (q_I/q_H)\partial_x I], \tag{40}$$

where

$$\Delta_H \equiv d\Delta/dH, \tag{41}$$

is obtained from Eq. 10, and

$$q_I \equiv \partial q/\partial I \quad \text{and} \quad q_H \equiv \partial q/\partial H, \tag{42}$$

are obtained from Eq. 9. Thus, we obtain

$$\partial_t q = \partial_x [D_{11} \partial_x q + D_{12} \partial_x I] \tag{43}$$

$$\partial_t I = \partial_x [D_{21} \partial_x q + D_{22} \partial_x I] \tag{44}$$

where

$$D_{11} = \frac{D_H}{\tau} \left[\frac{Q_s + \xi\tau Q}{Q + Q_s}\right] \tag{45}$$

$$D_{12} = -\frac{D_H}{\tau} \left[\frac{q_I Q_s + 2\eta\tau Q}{Q + Q_s}\right] \tag{46}$$

$$D_{21} = -\frac{D_H}{2} \left[\eta + \frac{\Delta_s}{Q + Q_s}(\tau^{-1} - \xi)\right] \tag{47}$$

and

$$D_{22} = D_H \left[\xi + \frac{\Delta_s}{Q + Q_s}\left(\frac{q_I}{2\tau} - 2\right)\right], \tag{48}$$

where

$$\tau = \tau(H) = \left.\frac{dq}{d\Delta}\right|_I = \frac{\dfrac{\partial q}{\partial H}}{\dfrac{\partial \Delta}{\partial H}} = \frac{1 + \dfrac{K_W}{H^2} + \dfrac{A_T \partial_{pH} \bar{r}_A + B_T \partial_{pH} \bar{r}_B}{H \ln 10}}{1 + \dfrac{D_{OH}}{D_H}\dfrac{K_W}{H^2} + \dfrac{D_B}{D_H}\dfrac{B_T \partial_{pH} \bar{r}_B}{H \ln 10}}, \tag{49}$$

is the association-dissociation time-scale factor introduced previously by Deem et al. (4). τ can be interpreted physically as a compliance ratio between charge gradient and flux at fixed ionic strength. The numerator expresses the local gradient of charge due to protons and charge species which rapidly and reversibly associate with protons. The denominator is proportional to the flux of mobile charges. Accordingly, τ has maxima

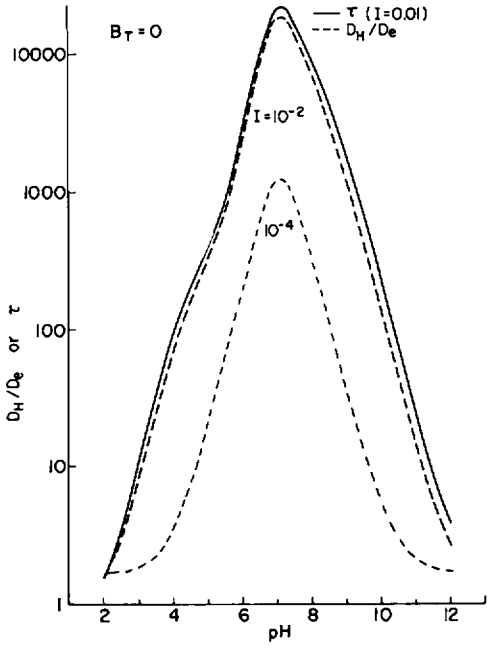

FIGURE 5

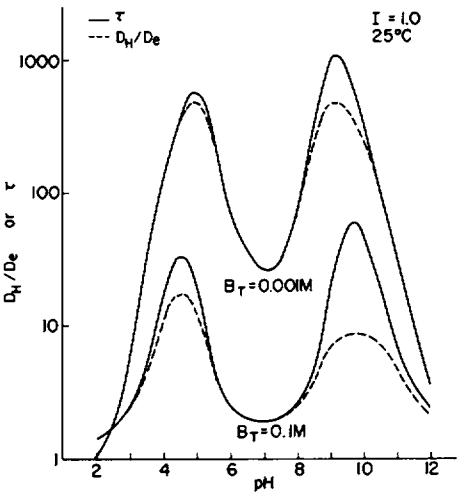

FIGURE 6

FIGURE 5 D_H/D_e vs. pH at various ionic strengths in the absence of mobile buffers (B_T = 0). Immobilized, 10% by weight, papain membrane. Solid curve is with I = 0.01 M. Diffusivities and other properties are given in Fig. 4.

FIGURE 6 D_H/D_e vs. pH at high ionic strength (I = 1.0 M) for an ideal phosphate buffer with B_T = 0.001 M and B_T = 0.1 M in an immobilized, 10% by weight, membrane. The buffer properties and diffusivities are given in Fig. 4.

at pH values where the increment in Δ is small (or weakly compliant) compared to the increment in q. Note Q, Δ_s, and Q_s are related to q and I through Eqs. 11, 20, and 21.

The mathematical properties of this set of conservation equations (e.g. stable vs. unstable equilibria, etc.) depends upon the species diffusion coefficients (e.g., ξ and η, etc.) and upon q_I and τ, which are derived from the titration curve of the particular protein. For example, if we consider an ideal electrolyte we set $\eta = 0$. Furthermore, for most proteins, the variation of the dissociation constants or titration curve with ionic strength is very small in a region of pH near the isoelectric point (Tanford, reference 16, section 30). Thus another simplification is obtained if we set $(\partial q/\partial I) \equiv 0$ giving

$$\partial_t q = \partial_x[D_e \partial_x q], \tag{50}$$

$$\partial_t I = \partial_x[D_{ss} \partial_x q + D_s \partial_x I], \tag{51}$$

where

$$D_e = \frac{D_H}{\tau}\left[\frac{1 + \tau Q/2I}{1 + D_H Q/2D_s I}\right], \tag{52}$$

and

$$D_{se} = -\frac{D_s}{2}\,\frac{q}{I}\left[\frac{1 - D_H/\tau D_s}{1 + D_H Q/2D_s I}\right]. \tag{53}$$

Within the present asymptotic description, one cannot set $I = 0$, for this implies $q_s^{(0)} = 0$ and thus $q^{(0)} = 0$. From Eq. 9 this requires H to be a specific constant and thus Q and Δ are constants, and their time and space derivatives vanish in this order.

Figs. 5, 6, and 7 show the pH dependence of τ and (D_H/D_e), Eq. 52, for $I = 1.0$, 10^{-2}, and 10^{-4} M and for various buffer concentrations, B_T. The value of τ is computed with $\bar{r}_A = \bar{r}_A(H, I)$, which we have taken as the titration curve of papain measured in homogeneous solution and where we have allowed the pK's to depend explicitly on the ionic strength as described by Tanford (16). One must be cautious of the homogeneous solution assumption if the proteins are chemically immobilized (Tanford [16], p. 524). In practice, one should use for $\bar{r}_A$ an effective titration curve obtained from direct measurements on the immobilized protein system. The explicit appearance of I in $\bar{r}_A$ is manifest in the small differences between the solid curve in Figs. 6 and 7. This supports the assumption made previously that one can omit the ionic strength dependence of τ in deriving effective diffusion coefficients.

Fig. 5 depicts the previous remark that electric field contributions to H^+ ion transport are cancelled at high salt concentrations. Figs. 6 and 7 show comparable results for ideal salts and an ideal phosphate buffer with $B_T = 10^{-3}$ and 10^{-1} M. We note that at a small but finite ionic strength (Fig. 7, $I = 0.01$) small amounts of buffer reduce D_H/D_e from τ. That is, buffers enhance the effects of intrinsic electric fields and reduce diffusion time scales, as shown in Fig. 8 of reference 4. At large ionic strength (Fig. 6, $I = 1.0$) there is little difference between τ and D_H/D_e except at pH values between the buffer pK's.

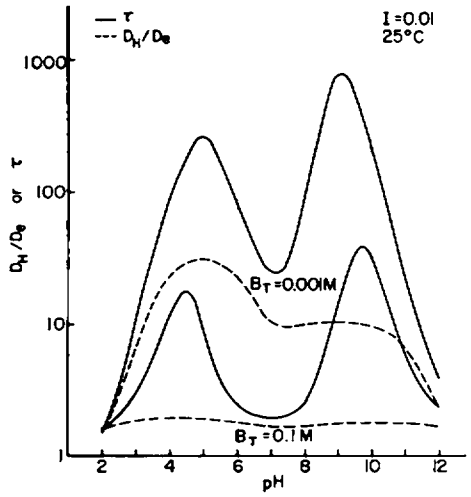

FIGURE 7 D_H/D_e vs. pH at low ionic strength ($I = 0.01$ M) for an ideal phosphate buffer with $B_T = 0.001$ M and $B_T = 0.1$ M in an immobilized, 10% by weight, membrane. The buffer properties and diffusivities are given in Fig. 4.

ELECTRIC FIELD

We substitute in Eq. 32 (superscripts suppressed) Δ_s obtained from Eq. 37*a* and obtain

$$E = \frac{(k_B T/e)}{(Q + Q_s)} \{(1 - \xi\tau)\partial_x \Delta + (2\eta - \xi q_I)\partial_x I\}. \tag{54}$$

For the ideal electrolyte ($\eta = 0$) and for a protein in the vicinity of its isoelectric point ($q_I = 0$)

$$E = (k_B T/e)(Q + Q_s)^{-1}[(1 - \tau(D_s/D_H))\partial_x \Delta]. \tag{55}$$

If $\tau D_s/D_H$ falls below 1.0, the electric field may reverse sign locally. For example, Fig. 7 shows that at large buffer concentrations, namely $B_T = 0.1$ M, then $\tau(10^{-7.2}) = 2.02$. KCl is nearly an "ideal" univalent electrolyte since $D_{Cl}/D_K = 1.04$. Since $D_K/D_H = 0.210$, then $D_s/D_H = 0.213$ and $(D_s/D_H)\tau(10^{-7.2}) = 0.430$. Thus, as the pH varies away from the broad minimum of the lower solid curve of Fig. 7 at pH = 7.2, τ increases and the electric field will reverse sign.

CONCLUSION

We have presented a theory for proton (H^+ ion) diffusion through immobilized protein membranes assuming a constant local buffer concentration B_T and local charge neutrality, and including the self-consistent coupling to mobile salts via intrinsic weak electric fields. The theoretical formulation resembles "ambipolar" diffusion in an electrolyte or a partially ionized gas and is based upon an asymptotic analysis where the electric field relaxation time $t_R = \bar{\lambda}_D^2/D_H$ is much smaller than the diffusion time $t_D = L_M^2/D_H$. For thin membranes ($L_M \approx 50$ Å) the theory shows that microscopic charge neutrality is valid on time scales greater than $\bar{t}_R = 10^{-6}$ if $I \approx B_T \approx 10^{-3}$ M.

We have derived a coupled pair of nonlinear diffusion equations and new effective diffusion coefficients, Eq. 43 through 48. These depend upon the titration curve of the fixed protein and the instantaneous distribution of salts and other mobile species, including H^+, OH^-, and pH buffer-related moieties in the membrane. If the buffer does not diffuse very rapidly, then B_T will be space-and-time-dependent. Thus, to obtain a complete description, a diffusion equation and boundary conditions for B_T must be included. Because charge neutrality prevails, an intrinsic electric field arises that depends locally on pH and the relative concentration of mobile buffers and salts. Large salt concentrations ($I > 0.1$ M) mitigate intrinsic electric field contributions to the transport of H^+ ions. Mobile buffers decrease pH relaxation times for protein membranes, consistent with experimental observations.

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