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The role of fixed and mobile buffers in the kinetics of proton movement

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We derive a simple expression for the effective diffusion coefficient of protons in Fick's second law, D^{eff}, when both spatially fixed, HF, and mobile, HM, buffers are present. These buffers are present at moderately high concentrations ($[F^{tot}]$, $[M^{tot}] > 1$ mM) in most biological systems. We consider only the case where the protonation reactions remain at equilibrium during the diffusion process. When the pH is to the alkaline side of the pK values of the fixed and mobile buffers ($[H^+] < K_F, K_M$), the effective diffusion coefficient of protons in Ficks second law is:

$$D^{eff} = \frac{D_H + \frac{D_{HM}[M^{tot}]}{K_M}}{1 + \frac{[M^{tot}]}{K_M} + \frac{[F^{tot}]}{K_F}}$$

where D_H is the diffusion coefficient of the protons free in the aqueous phase and D_{HM} is the diffusion coefficient of the mobile buffer. The equation illustrates three features of diffusion in a buffered system. Firstly, the effective diffusion coefficient of protons is always lower than the diffusion coefficient of free protons. Secondly, increasing the concentration of fixed buffers always decreases D^{eff}. Thirdly, increasing the concentration of mobile buffer can increase D^{eff} when fixed buffers are present.

Introduction

Spatially fixed buffers decrease the effective diffusion coefficient of buffered ions: the effective diffusion coefficient in Fick's second law is $D^{eff} = D/(1 + R)$, where D is the diffusion coefficient of the free ions in the aqueous phase and R is the ratio of bound-to-free ions (e.g., see page 327 of Ref. 1). The high concentration of fixed buffers for H^+ and OH^- in biological cells and organelles implies that R is large and that buffers should affect the kinetics of these ions' movement. For example, Polle and Junge [2] observed very slow relaxation of an alkalinization pulse in the narrow domain (width, 5 nm) between stacked thylakoid membranes; they [3] argued that fixed buffers in this narrow domain reduce the diffusion coefficient of OH^- by a factor of 10^4–10^5.

Mobile buffers, however, can counteract the effect of fixed buffers and enhance the effective diffusion coefficients of OH^- and H^+ when both types of buffer are present. Biological systems usually contain both fixed and mobile buffers. For example, the cytoplasm contains mobile buffers

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such as phosphate ($D \approx 10^{-9}$ m^2/s), the plasma and intracellular membranes contain mobile buffers such as phosphatidic acid ($D \approx 10^{-11}$ m^2/s), and both the aqueous and membrane phases contain fixed buffers such as proteins. We derive here a simple expression for the effective diffusion coefficient in Fick's second law when both fixed and mobile buffers are present.

We discuss here only the effect of buffers on the kinetics of proton movement. Other investigators have clearly discussed how mobile buffers enhance the steady-state flux of protons through an aqueous unstirred layer adjacent to a membrane [4] and from a source to a sink in a system of enzymes [5].

Analysis

We consider the kinetics of the proton movement described by Fick's second law. To simplify our analysis we assume the system is homogeneous *, diffusion coefficients are independent of position, diffusion occurs in one dimension **, activity coefficients are unity, the diffusion of H^+ is electroneutral because there is a high concentration of indifferent electrolyte, and the diffusion of OH^- may be ignored *** with respect to H^+. In the absence of buffers, Fick's second law is:

$$\frac{\partial[H^+]}{\partial t} = \frac{D_H \partial^2[H^+]}{\partial x^2} \tag{1}$$

* Our analysis may be extended simply to some inhomogeneous biological systems. For example, McLaughlin and Brown [11] considered the diffusion of an ion in an aqueous space between two membranes when the fixed buffer groups are bound to the membranes, and the membranes have a net negative charge. The surface concentration of a fixed buffer is divided by the width of the aqueous space to convert it into an effective three-dimensional buffer concentration and the value of the dissociation constant is divided by the Boltzmann term, $\exp(-zF\varphi(0)/RT)$, where z is the valence of the buffered ion and $\varphi(0)$ is the surface potential, to convert it from an apparent dissociation constant to an intrinsic one. The width of the aqueous space must be significantly smaller than the diffusion length for this treatment to be valid.

** When diffusion occurs in more than one dimension, the second-order spatial derivatives on the right-hand side of Eqns. 1, 2, 7, 10, 14 and 17 are replaced by the Laplacian, $\nabla^2 \equiv \nabla \cdot \nabla$.

*** We consider below an approximate expression that includes the effect of OH^- diffusion (Eqn. 19).

where D_H is the diffusion coefficient of protons in water ($D_H \approx 10^{-8}$ m^2/s). In the presence of buffers, HB_i (where B_i can be either a fixed, F_i, or mobile, M_i, buffer), we express Fick's second law as

$$\frac{\partial([H^+] + \Sigma_i[HB_i])}{\partial t} = \frac{D_H \partial^2[H^+]}{\partial x^2} + \frac{\Sigma_i D_i \partial^2[HB_i]}{\partial x^2} \tag{2}$$

This equation follows from the conservation of mass and Fick's first law, which states that the flux of a solute is proportional to the gradient of its concentration. Eqn. 2 states that the rate of change in the concentration of free and bound protons in an infinitesimal volume is equal to the divergence of the flux of these molecules into the volume. Note that the time derivative on the left-hand side of the equation includes both fixed and mobile buffers, but the second-order spatial derivative ** on the right hand side includes only the mobile buffers, for which $D_i > 0$.

Fixed buffers

Single pK

We first consider the case of one species of fixed buffer with a single dissociable group:

$$H^+ + F^- \rightleftarrows HF$$

We assume this protonation reaction is rapid compared to diffusion and may be considered at equilibrium †:

$$[H^+][F^-] = K_F[HF] \tag{3}$$

where K_F is the equilibrium dissociation constant. If we define

$$[F^{tot}] = [F^-] + [HF] \tag{4}$$

† The thickness of the 'reaction layer', the distance over which the equilibrium described by Eqn. 3 cannot be maintained, is about 1 nm when the buffer concentration is 0.1 M and about 10 nm when the buffer concentration is 0.001 M (e.g., Delahay [12], see pp. 87–95). Westerhof and Chen [13] have considered the diffusion of protons between a source and a sink separated by a distance less than the reaction layer.

then

$$[HF] = \frac{[F^{tot}][H^+]}{[H^+] + K_F} \tag{5}$$

To simplify the algebra, we assume $[H^+] \ll K_F$. In other words, we assume the pH is to the alkaline side of the pK. Eqn. 5 reduces now to

$$[HF] = \frac{[F^{tot}][H^+]}{K_F} \tag{6}$$

We insert Eqn. 6 into Eqn. 2 to obtain

$$\frac{\partial^2[H^+]}{\partial t^2} = \frac{D_{1a}\partial^2[H^+]}{\partial x^2} \tag{7}$$

where D_{1a} is the effective diffusion coefficient of the protons:

$$D_{1a} = \frac{D_H}{1 + \frac{[F^{tot}]}{K_F}} \tag{8}$$

This is the case considered by Crank [1], because $[F^{tot}]/K_F$ is the ratio of bound-to-free protons, $[HF]/[H^+]$, as illustrated by Eqn. 6. For example, if we assume that groups with a pK of 6 ($K_F = 10^{-6}$ M) are present at a concentration of $[F^{tot}] = 10^{-2}$ M on spatially fixed proteins, the effective diffusion coefficient is 10^4-fold lower than the diffusion coefficient of the free protons. According to the Einstein [6] relation, $x^2 = 2Dt$, it will take a proton $t = 100$ s rather than 10 ms to diffuse a distance $x = 10$ μm when the buffer is present.

Multiple pK values

Consider a more biologically relevant system in which the buffers are fixed proteins whose side chains have a broad spectrum of pK values. The slope of the titration curve, the buffer capacity β, is

$$\beta \equiv -\frac{d([H^+] + \Sigma_i[HF_i])}{d(pH)} \tag{9}$$

Inserting Eqn. 9 into Eqn. 2 we obtain

$$\frac{\partial[H^+]}{\partial t} = D_{1b}\frac{\partial^2[H^+]}{\partial x^2} \tag{10}$$

where

$$D_{1b} = D_H \frac{2.3[H^+]}{\beta} \tag{11}$$

Eqn. 11 is more general than Eqn. 8. The relationship between these two equations is illustrated by considering the limiting case where the buffers have only one dissociable group. The combination of Eqns. 5 and 9 yields

$$\beta = 2.3[H^+]\left(1 + \frac{[F^{tot}]K_F}{([H^+] + K_F)^2}\right) \tag{12}$$

If we assume, as before, that $[H^+] \ll K_F$, then combining Eqns. 11 and 12 yields Eqn. 8.

Fixed and mobile buffers

Single pK

We first consider the case where fixed and mobile buffers are present and each buffer has a single dissociable group. Eqn. 5 characterizes the fixed buffer and Eqn. 13 describes the mobile buffer if, as before, we assume that the reactions are essentially at equilibrium:

$$H^+ + M^- \rightleftarrows HM$$

$$[HM] = \frac{[M^{tot}][H^+]}{[H^+] + K_M} \tag{13}$$

where $[M^{tot}] = [M^-] + [HM]$. We assume that the pH is to the alkaline side of the pK values of both the mobile and fixed buffers, $[H^+] \ll K_M$, K_F, and insert Eqns. 5 and 13 into Eqn. 2 to obtain

$$\frac{\partial[H^+]}{\partial t} = D_{2a}\frac{\partial^2[H^+]}{\partial x^2} \tag{14}$$

where

$$D_{2a} = \frac{D_H + \frac{D_{HM}[M^{tot}]}{K_M}}{1 + \frac{[M^{tot}]}{K_M} + \frac{[F^{tot}]}{K_F}} \tag{15}$$

Note that when $[M^{tot}] = [F^{tot}] = 0$, Eqns. 14 and 15 reduce to Eqn. 1 and when $[M^{tot}] = 0$, they reduce to Eqns. 7 and 8. Eqn. 15 illustrates that fixed buffers always decrease the effective diffu-

sion coefficient, D_{2a}, but mobile buffers can either increase or decrease the value of D_{2a}. In the absence of fixed buffers, mobile buffers always decrease D_{2a}. For example, if $[M^{tot}]/K_M \gg 1$, then $D_{2a} \approx D_{HM}$. The maximum value of D_{HM} is about 10^{-9} m^2/s and is characteristic of a small molecule such as phosphate or bicarbonate. On the other hand, the value of D_H is about 10^{-8} m^2/s because protons diffuse by the Grotthus mechanism.

In the presence of fixed buffers, mobile buffers can partially compensate for the decrease in D_{2a} caused by the fixed molecules. Consider a numerical example: if pH = 7, $pK_F = pK_M = 6$, $[F^{tot}] = 10^{-2}$ M, $[M^{tot}] = 0$, then $D_{2a} = D_H/10^4$. However, if $[M^{tot}]$ is 10^{-3} M, D_{2a} increases to $D_{HM}/10 \approx D_H/10^2$.

Eqn. 15 can be simplified under certain conditions. If $pK_F = pK_M$, $[F^{tot}] > [M^{tot}]$ and $[F^{tot}]/K_F > 1$, then $D_{2a} \approx (D_{HM}/D_H)([M^{tot}]/[F^{tot}])D_H$. In other words, the effective diffusion coefficient is lower than the value in an unbuffered aqueous solution, D_H, by a factor $D_H/D_{HM} \approx 10$ because protons move mainly in the form of HM rather than free ions. It is reduced by a further factor $[F^{tot}]/[M^{tot}]$, which represents either the relative concentration of protons bound to fixed and mobile buffers or the relative time a single proton is bound to fixed and mobile buffers.

Multiple pK values

We now consider the case where both the mobile and fixed buffers have a distribution of pK values. We define a total buffer capacity:

$$\beta^{tot} = \Sigma_i \beta_i \tag{16}$$

where β_i is defined in analogy with Eqn. 9 for both the fixed and mobile buffers. It follows from Eqn. 2 that

$$\frac{\partial [H^+]}{\partial t} = \frac{D_{2b}\, \partial^2 [H^+]}{\partial x^2} \tag{17}$$

where

$$D_{2b} = \frac{2.3[H^+]D_H}{\beta^{tot}} + \frac{\Sigma_i D_i \beta_i}{\beta^{tot}} \tag{18}$$

Eqn. 18 describes more generally than Eqn. 15 the effect of fixed and mobile buffers on the effective diffusion coefficient of protons. Both equations allow us to draw three conclusions. Firstly, when buffers are present, the effective diffusion coefficient, D_{2b} in Eqn. 18, is always less than the free diffusion coefficient of protons, D_H. Secondly, fixed buffers always decrease D_{2b}. Thirdly, mobile buffers blunt this decrease; the buffer with the largest product $D_i\beta_i$ has the largest effect.

In the stroma of chloroplasts or the matrix of mitochondria the pH is alkaline and we expect the relaxation of a pH profile to be dominated by OH^- rather than by H^+ [3]. Around neutral pH, where both H^+ and OH^- contribute to the relaxation, the analysis is complicated. However, if we assume a high curvature for the initial pH profile, i.e.,

$$\frac{\partial^2 [H^+]}{\partial x^2} \gg \frac{1}{[H^+]}\left[\frac{\partial [H^+]}{\partial x}\right]^2$$

Eqn. 18 takes an approximate form:

$$D^{eff} \approx D_H\left[\frac{2.3[H^+]}{\beta^{tot}}\right] + D_{OH}\left[\frac{2.3 \cdot 10^{-14}}{\beta^{tot}[H^+]}\right] + \frac{\Sigma_i D_i \beta_i}{\beta^{tot}} \tag{19}$$

where D_{OH} denotes the diffusion coefficient of free OH^- and the other parameters are defined above.

Discussion

The analysis above is simple, but we cannot find a similar quantitative discussion in the literature. Other investigators have recognized that mobile buffers can reduce equilibration times for protons when fixed buffers are present. Engasser and Horvath [5], for example, discussed this phenomenon when they considered qualitatively the results of Bishop and Richards [7]. These investigators found that the acid-base titration of crystalline β-lactoglobulin crosslinked with glutaraldehyde took several hours to reach the endpoint, but addition of a small amount of acetate to the mixture reduced the equilibration time to 2 or 3 min. Engasser and Horvath [5] concluded that "buffer-facilitated proton transport can have profound significance in reducing the time needed to achieve equilibrium when the rate of equilibration is limited by the diffusion of H^+". We agree.

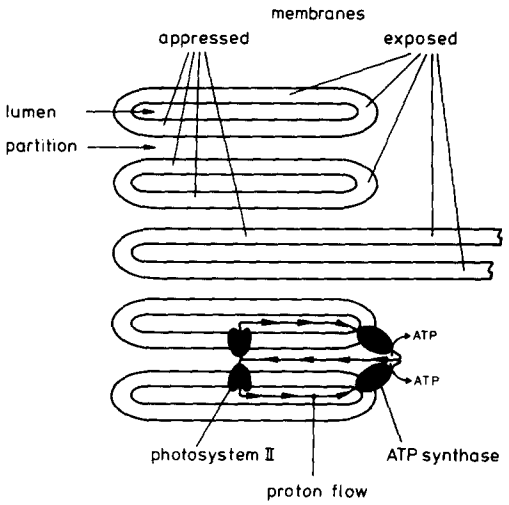

Fig. 1. Diagram illustrating the circulation of protons in thylakoids (see Ref. 3).

We consider one biologically relevant example of the effect of fixed and mobile buffers on the kinetics of proton movement. As illustrated in Fig. 1, protons cycle between Photosystem II, a light-driven proton pump, and ATP synthases in thylakoids [3]. The alkaline partition region and the acidic lumen both contain high concentrations of buffers [3,8–10]. If we assume the pH of the partition region is about 8, it is apparent from Eqn. 19 that the diffusion of OH^- is about two orders of magnitude more important than the diffusion of H^+ in this region. The buffer capacity of the partition region is at least $2 \cdot 10^{-2}$ M per pH at pH = 8 [3]. It is determined mainly by large protein complexes, which we assume have a diffusion coefficient of $D_{\text{protein}} = 10^{-13}$ m^2/s. We assume that the pK of inorganic phosphate is 7, its diffusion coefficient is $D_{\text{phosphate}} = 10^{-9}$ m^2/s, and its concentration in the partition region is 10^{-3} M. Eqn. 12 indicates that phosphate contributes only about 1% of the total buffer capacity at pH 8. That is, $\beta_{\text{protein}} \approx 0.99\ \beta^{\text{tot}}$ and $\beta_{\text{phosphate}} \approx 0.01\ \beta^{\text{tot}}$. However, Eqn. 19 illustrates that this concentration of phosphate has a large influence on the effective diffusion coefficient:

$$D^{\text{eff}} \approx D_{\text{OH}}(2.3 \cdot 10^{-6}/2 \cdot 10^{-2}) + D_{\text{phosphate}}(0.01) + D_{\text{protein}}(0.99)$$

$$\approx 10 \cdot 10^{-13} + 100 \cdot 10^{-13} + 10^{-13}\ \text{m}^2/\text{s}.$$

In the absence of phosphate ions the proteins are predicted to reduce the effective diffusion coefficient by at least four orders of magnitude from 10^{-8} to 10^{-12} m^2/s, because the term multiplying D_{OH} in Eqn. 19 is about 0.0001. This prediction agrees with the experimental results [3]. The addition of 10^{-3} M phosphate to the partition region should increase the effective diffusion coefficient by an order of magnitude even though it contributes only 1% to the buffer capacity of this region.

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