



Mechanism of long-range proton translocation along biological membranes



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ABSTRACT

Recent experiments suggest that protons can travel along biological membranes up to tens of micrometers, but the mechanism of transport is unknown. To explain such a long-range proton translocation we describe a model that takes into account the coupled bulk diffusion that accompanies the migration of protons on the surface. We show that protons diffusing at or near the surface before equilibrating with the bulk desorb and re-adsorb at the surface thousands of times, giving rise to a power-law desorption kinetics. As a result, the decay of the surface protons occurs very slowly, allowing for establishing local gradient and local exchange, as was envisioned in the early local models of biological energy transduction.

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1. Introduction

Proton exchange between proton pumps and ATP synthase plays a key role in biological energy transduction. Despite a half-century-long history of chemiosmotic theory, the exact mechanism of proton transport in the membrane proton circuits still remains a subject of intense debate, rendering the celebrated theory incomplete. The debated issue is the mode of exchange – global or local – as presented by several competing models [1–6].

In the model proposed by Mitchell [1,2], the protons released on the outer side of the membrane by proton pumps equilibrate with the bulk, and it is the equilibrium difference in pH on the two sides of the membrane, along with the membrane potential generated by the proton gradient common for the whole organelle, that act on the ATP synthase. However, as was originally pointed out by Williams [3,4], this mechanism is not most efficient because protons released to the bulk irreversibly lose part of their free energy. In Williams' model, protons always remain inside the membrane, providing for local exchange and ensuring maximum efficiency of energy transduction. However, it was not clear what would keep protons inside the membrane (see [Supplementary material](#) for additional comments). Kell [5] developed an alternative view of local coupling by introducing an interphase, which assumes a barrier between the membrane and the bulk solution and also special pathways of efficient proton lateral movement via chains of “struc-

tured” water molecules. Skulachev [6] argued that if the local coupling were true, ATP synthesis would be possible in open systems, which had never been observed. Instead, he combined Mitchell's delocalized mechanism with a local contribution due to highly curved structure of the inner mitochondrial membrane where proton generators and consumers are closely spaced on different sides of cristae. While this type of local coupling may indeed be an essential part to the driving force, Skulachev's argument cannot be used to dismiss the Williams/Kell coupling type, for in open systems the opposite sides of the membrane are not isolated from each other, hence protons can leak between them around the edge [7,8] on the same time-scale or faster than ATP synthase turnover. The easiest way to prevent this is to use a closed membrane isolating inside and outside, as in the original chemiosmotic theory; however, the closed surface by itself does not mean that the coupling is global via equilibrated protons.

Numerous studies of the past two decades [7–24] indicate that the local model is more likely to be realized in real cells. The experiments, while often not unambiguous [23–26], did reveal however a puzzling property of protons migrating along the membrane surface – they appear to remain on the surface for too long, making surface transport surprisingly long-distant, up to tens of micrometers. This long apparent surface dwell-time – up to hundreds of milliseconds – calls for an unusually deep potential well that would keep protons at the surface. A simple estimate by the transition-state theory (TST) gives the potential barrier of about 30 RT for the dwell-time observed. The mechanism of such significant proton affinity to the surface is hard to rationalize. Moreover, as was recently reported [9], the retention time does not appear to

Abbreviation: TST, transition-state theory

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depend on the properties of the surface charged groups that could potentially retain the protons, adding to the puzzle. (For further estimates of the barrier and discussion of proton retention at the interface, see [Supplementary material](#).)

Here we show that the puzzling observations of proton migration on the micrometer scale along the membranes and the retention time up to a second can be explained by taking into account the coupled bulk diffusion which accompanies migration of protons on the surface [14,15]. The apparent long-time retention of protons at the interface surface is not due to a deep potential well or high- pK_a ionized groups, but rather due to compensation of the surface depletion by return of the released protons and their re-adsorption by the surface. This back reaction results in a power-law decay kinetics of the surface protons and explains both their long apparent dwell-time on the surface and corresponding long-distance lateral migration, and their high apparent surface diffusivity.

2. Results and discussion

If a surface attracts and retains protons, whatever the microscopic mechanism is, its proton affinity can be characterized by the equilibrium condition

$$\sigma_{\text{eq}} = L_0 n_{\text{eq}} \quad (1)$$

where σ_{eq} and $n_{\text{eq}} = 10^{-\text{pH}}$ are equilibrium surface and bulk concentrations and L_0 is the equilibrium constant with dimension of length. As we will see, this is the distance over which surface and bulk protons are coupled. The equilibrium surface concentration can be expressed by equation

$$\sigma_{\text{eq}} = n_{\text{eq}} \exp(U/RT) d \quad (2)$$

where d is the width of the interfacial water layer where the protons are retained and U is the well depth that represents the difference in proton free energies between the bulk and the surface layer (no barrier from the bulk side is assumed). Eq. (2) expresses the fact that attraction of protons to surface increases acidity (decreases pH) of the interfacial layer [17]. Combining Eqs. (1) and (2), we derive a formula for the equilibrium constant,

$$L_0 = d \exp(U/RT) \quad (3)$$

Assuming $d = 1$ nm and $U = 12 RT$, we obtain $L_0 = 160$ μm . This is the key point where the dimension of micrometer size appears. If the surface retains protons due to ionized groups, then L_0 is expressed in terms of their surface density and pK_a [27,28]; a similar estimate, $L_0 = 170$ μm , was given [27] for un-buffered solutions. As will be seen below, it is diffusion over this long distance in the bulk that sets the relevant time-scale for proton escape from the surface.

In the above estimates, we used an order-of-magnitude value for d and a median U value of those cited in the [Supplementary material](#). If we use the lowest barrier, $U = 5 RT$, and a thinner interfacial layer, $d = 0.6$ nm, corresponding to two hydrogen bonds, we obtain $L_0 = 0.1$ μm , which is still macroscopically long and is compatible with experimental data of Heberle et al. [7] and Alexiev et al. [8], where proton diffusion was observed at purple membrane sheets of 0.6 and 0.25 μm , respectively.

The kinetic model is formulated as follows. We consider a typical experimental setup: Excess protons are released on the surface at time $t = 0$ by a source of finite size r_0 and are observed at a spot on the surface at distance $r \gg r_0$. In order to understand how the excess protons are distributed over the surface and in the bulk, and how this distribution is changing with time, one needs to solve coupled surface and bulk diffusion equations [15] for $\sigma(r, t)$ and $n(r, z, t)$ (see Section 3). The analysis shows that there are two types of

long-time decay kinetics of the excess protons on the surface: exponential and power-law, depending on a single parameter ν , see Eq. (6), which is a combination of L_0 , the bulk diffusion coefficient D_b , and the desorption rate constant k_{off} . The two limiting cases are referred to as slow and fast exchange between the surface and bulk protons [27,28]. In these two regimes, the mechanisms of proton transfer along the surface are fundamentally different. Below, we discuss these two limiting cases separately, using simplified models, which demonstrate explicitly the essential points and accurately follow the exact solution [15].

Two populations of excess protons are considered: total surface population $p_s(t)$, i.e. population of the interfacial layer, and total bulk population $p_b(t)$. The interfacial layer has width d , as above. The bulk part can be also considered as a layer whose width is increasing with time owing to diffusion normal to the surface,

$$L_b(t) \propto \sqrt{D_b t}.$$

The exact analysis shows that

$$L_b(t) = \sqrt{\pi D_b t}.$$

The rate with which protons are exchanged between the surface and the bulk can be fast or slow with respect to diffusion. In the fast-exchange regime, thermal equilibrium is established between the two layers at every moment of time (it will be clear later that this is the most realistic case). The two populations are normalized as

$$1 = p_s + p_b = p_s + p_s e^{-U/RT} (L_b/d) \quad (4)$$

hence, the total surface population is approximately given by

$$p_s(t) = [1 + (L_b(t)/L_0)]^{-1} \quad (5)$$

which differs insignificantly from a rigorous equation [15]. The relation between p_s and p_b used in Eq. (4) is determined by the relative energy of the layers, U , and by the number of sub-states in each layer, which is proportional to its size, i.e. d for the surface layer and L_b for the bulk layer. The second term in brackets of Eq. (5) leads to depletion of the surface population with time because of diffusion normal to the surface and resulting increase of L_b . It is clear that the surface population p_s will be half-depleted over the time interval equal to that of diffusion in the bulk over distance $L_b = L_0$; that is, the effective dwell time will be of the order of $t_0 = L_0^2/D_b$. This time is quite long because of large L_0 . Obviously, the key here is the fast exchange, i.e. fast forward and back reactions, between layers s and b , compared to the diffusion equilibration time t_0 . This condition is equivalent to [see Section 3 and Ref. [28]]

$$\nu \equiv k_{\text{off}} t_0 \gg 1 \quad (6)$$

Here, we use diffusion coefficient D_b assuming that diffusion normal to surface at the surface layer is of the same order as in the bulk; in fact, there may be some small variations of this parameter [29,30], however, it cannot change the qualitative picture. If we assume diffusion-controlled escape from the surface (the Kramers limit of TST), then the proton hopping time is simply the time d^2/D_b needed for a proton to diffuse across the interfacial layer. Multiplying the corresponding rate by the Boltzmann factor and using Eq. (3), we obtain $k_{\text{off}} = D_b/L_0 d$, which results in an approximate estimate (based on $D_b = 9.3 \times 10^{-5}$ cm^2/s and $L_0 = 160$ μm) $k_{\text{off}}^{-1} = 10$ μs or shorter. A similar estimate is obtained when the protons are retained at the surface by ionized groups [15,27]. We see that, since $d \ll L_0$ by a factor $\exp(U/RT)$, the condition of fast equilibrium, Eq. (6), is indeed satisfied with a large margin: For $U = 12$ and $5 RT$ used above, we obtain $\nu \sim 10^5$ and 10^2 , respectively. Thus, the fast exchange case considered above is most realistic and directly applicable to the membrane systems studied in Refs. [7–9].

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