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Research Report

Electrostatic Distribution of H^+ in Mitochondria

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Abstract

This paper presents an analysis of the relationship between the electrostatic and osmotic contributions to the proto-motive force that represents the energetic intermediary for ATP synthesis according to the chemiosmotic theory. Particularly, it is shown that there is an additional constraint beyond those usually considered relating the transmembrane potential and the positive charge density composed of H^+ and other positive ions. The results of computation indicate that commonly quoted charge densities are not sufficient to produce frequently cited transmembrane potentials.

1 Introduction

The production of ATP by ATP synthase requires an energetic intermediary. However, searches proved chemical intermediaries to be elusive. Ultimately,

the proper intermediary was found to be electrostatic rather than chemical. [1][2][3] The energy is transferred to ATPase via an electrostatic potential gradient across the inner membrane of the mitochondria which drives H^+ ions through the core of the transmembrane ATPase. The source of the potential gradient is a net accumulation of H^+ on the cytosol side of the inner membrane. Therefore, the form of the chemical potential, usually referred to as "proto-motive force" when expressed in units of voltage, is composed of two parts:

$$\Delta p = \frac{\Delta \mu_{H^+}}{e} = \Delta \psi + \frac{kT}{e} \Delta \ln n_0, \tag{1}$$

where e is the electronic charge, μ_{H^+} is the chemical potential of the hydronium ions, p is the proto-motive force, ψ is the electrostatic potential, n_0 is the number density of hydronium ions, and Δ is the difference across the membrane. The electrostatic constraints, however, must be satisfied. Particularly, $\nabla^2 \psi = -\rho/\epsilon$ must be satisfied along with $\Delta \mu_{H^+} = 0$ in the inter-membrane space. These constraints imply

$$\nabla^2 \psi = \rho_0 e^{-\frac{e\psi}{kT}}, \tag{2}$$

which is usually called the "Poisson-Boltzmann equation." This performs a role similar to that of a "stoichiometric" constraint between ψ and $n(\vec{x}) = n_0 e^{-\frac{e\psi}{kT}}$.

An analogy between the inner mitochondrial membrane and an electrical capacitor is frequently offered. However the analogy is not strictly extended. Within a conductive capacitive plate, $\vec{E} = -\vec{\nabla}\psi = \vec{0}$, otherwise the electric field would drive a current of charge carriers through the conductor. Since

Gauss' law implies that there cannot be any non-zero charge density interior to the conductor without producing some $\vec{E} \neq \vec{0}$, the charges are all concentrated on the surface of the conductor in such a way that the surface is equipotential (the charges arrange themselves so that $-\vec{\nabla}\psi = \vec{0}$ on the surface). This implies that the charges are strongly localized on the surface of the conductive plates of of a capacitor. However, the more elementary biochemical literature usually treats pH^+ as fairly uniformly distributed throughout the mitochondrial inter-membrane space. Discussions remain focused primarily on chemical processes.

In the context of chemiosmotic theory and on the length scales appropriate to mitochondria, the capacitor analogy should be extended to include thermodynamic effects. The surface charge invades the inter-membrane space to some characteristic depth due to thermal excitation. This is accounted for by $\vec{\nabla}\mu_{H^+} = \vec{0}$, so that $\vec{E} = -\vec{\nabla}\psi = \frac{kT}{e}\vec{\nabla}\ln n(\vec{x})$. The equivalent to the condition of conductor-like electrostatic screening in the inter-membrane space is that $\vec{E} \to \vec{0}$ as the distance from the membrane increases. Together with a specification of geometry, this is sufficient to determine a characteristic thickness of the H^+ region as well as a value for n_0 given a transmembrane potential $\Delta\psi$, membrane thickness L, and dielectric constants κ_m for the membrane, and κ_{H_2O} in the inter-membrane space.

The literature provides reviews of measurements of $\Delta\psi$ and pH^+, [3][4] including some focused on related issues such as complications of the stoichiometry [5] [6] as well as analysis of chemical probes [6][7][8], including recent measurements with higher spacial resolution achieved with confocal

microscopy.[9] Recent analysis and reviews of charge distributions in the vicinity of the surface of membranes generally discuss two-component Z-Z electrolytic solutions [7][10] [11][12] generally employ the Gouy-Chapman [13][14] theory, augmented by Langmuir absorption which was applied to the Gouy-Chapman theory by Stern.[15] However, balanced or neutral Z-Z electrolytes do not describe the non-neutral ionic charge separations across mitochondrial membranes as required by chemiosmotic theory.

Robertson's application of Gouy-Chapman-Stern theory was to the question of how significant the contribution of the binding of 1-anilinonapthalene-8-sulfonate (ANS) to putative negative surface charges[16] is, and whether instead the ANS was concentrating in the predominantly negatively charged mitochondrial matrix. They concluded that there were no definitive results on a measure of such a surface charge.

This paper presents a more detailed analysis of the constraints outlined above. In the case of a simple planar geometry, relationships between n_0 and $\Delta\psi$ are derived. We compare these results with commonly cited parameters.

2 Electrostatic Constraints

This section presents a derivation of the distribution of H^+ ions. This begins with equations governing simple electrostatics in the inter-membrane space. The membrane is assumed to be a simple plane with an extent large compared to the scale of variation in the density of H^+. So $\vec{\nabla} = \hat{z}d/dz$, with no variations in directions parallel to the $x - y$ plane being considered. In this

geometry, the electrostatic equations are

$$-\frac{d\psi}{dz} = E, \tag{3}$$

$$\frac{dE}{dz} = \frac{\rho}{\epsilon_{\mathrm{H_2O}}}, \tag{4}$$

where ψ is the electrostatic potential, $\epsilon_{\mathrm{H_2O}}$ is the dielectric permittivity of the inter-membrane space, and E is the electric field. The chemical potential satisfies

$$\mu = e\psi + kT \ln \rho \tag{5}$$

which must be a constant throughout the inter-membrane space. It follows that

$$\rho = \rho_0 e^{-\frac{e\psi}{kT}}, \tag{6}$$

for some constant ρ_0. Define

$$\zeta = z/z_0, \tag{7}$$

$$\Psi = \frac{e\psi}{kT}, \tag{8}$$

$$\mathcal{E} = \frac{z_0 eE}{kT}, \tag{9}$$

$$r = \frac{z_0^2 e\rho}{\epsilon_{\mathrm{H_2O}} kT}, \tag{10}$$

$$r_0 = \frac{z_0^2 e\rho_0}{\epsilon_{\mathrm{H_2O}} kT}, \tag{11}$$

for some length scale z_0. Then

$$-\frac{d\Psi}{d\zeta} = \mathcal{E}, \tag{12}$$

$$\frac{d\mathcal{E}}{d\zeta} = r_0 e^{-\Psi}. \tag{13}$$

Further, the definition of r_0 may be constrained by choosing $\Psi(0) = 0$. This is an arbitrary choice since r_0 would be simply rescaled by a factor of $e^{-\Psi(0)}$. Then ρ_0 is the maximum H^+ concentration density at the mitochondrial inner-membrane surface ($z = 0$). The problem is then to find a value for r_0 that corresponds to an electrostatic screened state. If r_0 is too small, then there is not enough charge and E does not go to zero for large ζ, while if r_0 is too large, then there is too much charge, and E actually reverses as ζ increases. This is explicitly seen in the integration of the differential equations. Since z_0 remains undetermined, it may be selected so that $\mathcal{E}(0) = -1$.

The coupled equations combine to yield

$$\frac{d^2\Psi}{d\zeta^2} = -r_0 e^{-\Psi} = \frac{d\Psi}{d\zeta}\frac{d}{d\Psi}\left(\frac{d\Psi}{d\zeta}\right) = \frac{1}{2}\frac{d}{d\Psi}\left[\left(\frac{d\Psi}{d\zeta}\right)^2\right]$$

which has the simple solution

$$\left(\frac{d\Psi}{d\zeta}\right)^2 = 2r_0 e^{-\Psi} + C_1 \tag{14}$$

for some integrating constant C_1. Applying boundary conditions

$$\begin{aligned} \frac{d\Psi}{d\zeta}(0^+) &= -\mathcal{E}(0^+) = 1 \\ \Psi(0) &= 0 \end{aligned}$$

it follows that

$$C_1 = 1 - 2r_0. \tag{15}$$

Defining

$$u = e^{\Psi} \tag{16}$$

the first integral becomes

$$\left(\frac{du}{d\zeta}\right)^2 = 2r_0 u + C_1 u^2. \tag{17}$$

Integration of this breaks down into three cases: $C_1 < 0$, $C_1 = 0$, and $C_1 > 0$.

Considering the case where $C_1 > 0$ first, the expression on the right hand side of the equation becomes

$$C_1\left(u^2 + \frac{2r_0}{C_1}u\right) = C_1\left(u + \frac{r_0}{C_1}\right)^2 - C_1\frac{r_0^2}{C_1^2}.$$

Defining

$$\frac{r_0}{C_1}\cosh v = u + \frac{r_0}{C_1}$$

The equation integrates to yield

$$\zeta + C_2 = \int \frac{du}{\sqrt{2r_0 u + C_1 u^2}} = \frac{v}{\sqrt{C_1}},$$

so that

$$u = e^{\Psi} = \frac{r_0}{C_1}\left\{\cosh\left[\sqrt{C_1}(\zeta + C_2)\right] - 1\right\}. \tag{18}$$

This diverges as $\zeta \to \infty$, and so is discarded. This corresponds to the underscreened condition.

The next case to consider is $C_1 < 0$. Define $k_1 = -C_1$. Then the right hand side of the equation becomes

$$-k_1 u^2 + 2r_0 u = -k_1\left(u^2 - \frac{2r_0}{k_1}u\right) = -k_1\left(u - \frac{r_0}{k_1}\right)^2 + k_1\frac{r_0^2}{k_1^2}.$$

Defining

$$\frac{r_0}{k_1}\cos v = u - \frac{r_0}{k_1}$$

the equation integrates to yield

$$\zeta + C_2 = \int \frac{du}{\sqrt{2r_0 u - k_1 u^2}} = \pm \frac{v}{\sqrt{k_1}},$$

so that the solution is

$$u = e^{\Psi} = \frac{r_0}{k_1} \left\{ \cos \left[\sqrt{k_1} (\zeta + C_2) \right] + 1 \right\}. \tag{19}$$

This solution oscillates, and does not satisfy the boundary conditions as $\zeta \rightarrow \infty$. This corresponds to the overscreened condition. However, this solution may be appropriate for finite inter-membrane widths in the crystae folds. In this case, C_1 would be selected by the width of the inter-membrane space $\Delta\zeta$, which determines r_0. The effect of the folds would then be to support even greater densities of H^+ ions per generated trans-membrane potential (capacitance), possibly an added evolutionary adaptive benefit. However, this refinement will not be considered further here.

The final condition is $C_1 = 0$, which implies $r_0 = 1/2$. In this case,

$$\zeta + C_2 = \int \frac{du}{\sqrt{u}} = 2\sqrt{u}$$

The solution is thus

$$\Psi = 2 \ln \left(\frac{\zeta}{2} + 1 \right), \tag{20}$$

$$\mathcal{E} = \frac{-2}{2 + \zeta}, \tag{21}$$

$$r = r_0 \left(\frac{\zeta}{2} + 1 \right)^{-2}. \tag{22}$$

Further, if $\mathcal{E}(0^+) = -1$, it follows

$$z_0 = -\frac{kT}{eE(0^+)}.$$

This z_0 then corresponds to the characteristic screening depth or thickness of the H^+ layer. Inside the membrane, the electric field is assumed to be determined simply by the transmembrane potential V_0 and the membrane thickness L. Further, for an infinitely thin "pillbox" $\oint \hat{n} \cdot \vec{D} da = \oint \epsilon \hat{n} \cdot \vec{E} da = q_{\text{free}} \to 0$, so that

$$E(0^+) = -\frac{\epsilon_m V_0}{\epsilon_{H_2O} L}, \tag{23}$$

where ϵ_m is the dielectric permittivity of the membrane. The values of ρ_0 and z_0 are then

$$z_0 = \frac{kT}{e} \frac{\epsilon_{H_2O}}{\epsilon_m} \frac{L}{V_0}, \tag{24}$$

$$\rho_0 = \frac{e r_0}{kT} \frac{\epsilon_m^2}{\epsilon_{H_2O}} \frac{V_0^2}{L^2}. \tag{25}$$

Lastly, integration of equation 4 over $z = 0$ to ∞ yields the surface charge density of the inner membrane

$$\sigma = \int_0^\infty \rho(z) dz = -\epsilon_{H_2O} E(0+), \tag{26}$$

which is just Gauss' law applied to a "Gaussian pillbox" on a small surface element. This further implies

$$\sigma = z_0 \rho_0. \tag{27}$$

The preceding demonstrates that the number density $n_0 = \rho_0/e$ of H^+ ions is dependent on the transmembrane potential $\Delta\psi = V_0$. This implies that the transmembrane contribution $\Delta\psi$ and the osmotic contribution $\frac{kT}{e} \Delta \ln n_0$ to the proto-motive force

$$\Delta p = \frac{\Delta \mu_{H^+}}{e} = \Delta\psi + \frac{kT}{e} \Delta \ln n_0,$$

are not independent, but rather are strictly related.

3 Results

The constants used here to compare the preceding results with commonly cited figures were chosen to reflect a compromise between laboratory and physiological conditions. These constants are expressed in table I. The figure

Table I: Constants

Constant	Value
$\epsilon_0[C^2N^{-1}m^{-2}]$	8.85×10^{-12}
ϵ_m	$\kappa_m\epsilon_0$
$\epsilon_{\mathrm{H_2O}}$	$\kappa_{\mathrm{H_2O}}\epsilon_0$
$\kappa_{\mathrm{H_2O}}$	78.5
$T[K]$	237
$k[JK^{-1}]$	1.38×10^{-23}
$e[C]$	1.6×10^{-19}

for $\kappa_{\mathrm{H_2O}}$ was cited by Bokris and Reddy[17] in the temprature ranges considered here (77°F). The remaining physical constants were cited in Resnick and Halliday.[18] The selections of parameters describing the mitochondrial inner membrane seem to be typical of the literature, and are listed in table II. The membrane thicknesses and potentials are cited in several texts.[2] [3]

Table II: Assumed Mitochondria Parameters

Parameter	Value
$L[m]$	6.6×10^{-9}
$V_0[V]$	0.14
κ_m	$1 \leq \kappa_m \leq 10$

The dielectric permitivity of the membrane was cited from Mathews et al.[19]

The resulting computed characteristic depths z_0 and H^+ concentrations are listed in Table III. It is seen that the characteristic depths, as a funciton of

Table III: Predicted Mitochondrial H^+ Distribution Characteristics

Parameter	$\kappa_m = 1$	$\kappa_m = 2$	$\kappa_m = 10$
$z_0[m]$	75.2×10^{-9}	37.6×10^{-9}	7.52×10^{-9}
$\rho_0/e[m^{-3}]$	7.75×10^{21}	3.10×10^{22}	7.755×10^{23}
$\rho_0/e[M]$	1.29×10^{-5}	5.15×10^{-5}	1.29×10^{-3}
pH^+	4.89	4.28	2.89

membrane dielectric permitivity, range from numbers that are comparable to the membrane thickness to numbers that would fill the inter-membrane space. Further, the peak $[H^+]$ concentrations are much higher than commonly expected and cited. Since the chemical potential is a constant in the inter-membrane space, it is this density that would be representative of the osmotic force. The results corresponding to the smallest membrane dielectric permittivities κ_m seem to be closest to commonly cited values.

4 Discussion

The model presented here contains simplifications including some that are questionable. For example, a matrix concentration of nearly $0.5mg$ of protein per $1\mu l$ inside the inner membrane[3] suggests a complex chemistry including the possibility of buffering effects, which modifies the chemical potential. The details of those effects were not considered. Further, the effects of surface adsorption, surface charges, and Donnan potentials have also not been explicitly considered.

However, the key assumptions of the model developed here are motivated by very fundamental physical requirements. Particularly, charge is required to produce electric fields such as the those that generate the mitochondrial transmembrane potentials. The characteristic length z_0 is simply the distance that the thermal energy kT can provide to a charge e against a field $E(0^+)$, yielding $z_0 eE(0^+) \sim kT$. Further, the characteristic distance that the surface charge density $\sigma = \epsilon_{\mathrm{H_2O}} E(0^+)$, required by Gauss' law, can be extended into the inter-membrane space is z_0, yielding an effective density $\rho_0 \sim \sigma / z_0$. While it would not be expected that such a simple model would be exact given the crude estimates of the dielectric constants, simplified geometry, and variability in the individual physiology of mitochondria, a much larger $[\mathrm{H}^+]$ concentration is required to produce the commonly cited potentials than is usually posited.

Other problems with the commonly quoted numbers are as follows. Mitochondria sizes vary, but could be typified as cylindrical, with a radius of about $0.5\mu m$ and a length of about $3\mu m$. This implies a volume of $2.356 \times 10^{-18} m^3$. The inter-membrane space is composed of a number of very thin, ramified folds. Even if the entire inter-membrane space occupied a volumetric fraction $(1/5)^3$ of the entire mitochondrion, this would represent less than 1% of the entire volume. Given a cited $\Delta \mathrm{pH}^+ = 0.7$ less than a neutral 7,[3] the expected concentration would be $[\mathrm{H}^+]_{\mathrm{out}} = 5.01 \times 10^{-7} M = 3.02 \times 10^{20} m^{-3}$, so that the total expected number of protons expected in a mitochondrion would be about 7. On any particular crystus, or inner membrane fold, the number of protons could be best described as "occasional" (reminiscent of

homeopathy). While the conduction of protons through H_3O^+ does involve quantum tunneling, the rate-limiting step to that conduction is the consistent field-induced alignment with the acceptor H_2O lone-pairs.[17] This implies that the protons will be fairly well localized (no extended proton "orbitals" or band structures). Statistical notions such as chemical potential, as well as the possibility of computing a meaningful transmembrane potential $\Delta\psi$, are tenuous at such low $[H^+]$. Transmembrane potentials indicated by spectroscopic shifts of intrinsic probes such as carotenoids and other extrinsic probes such as lipophilic dyes, as well as induced gradients of ionic probes such as KCl demonstrate that H^+ ions are not occasional. More direct evidence of the persistent presence of $[H^+]$ has been seen in the fluorescence quenching of some acridine dyes, such as phenosafranine, cyanine, oxonols, and 1,9-aminoacridine.[3]

Robertson showed that experimental data interpreted in the context of the Gouy-Chapman-Stern model that binding of ANS to surface charges were not indicated.[7] However, it is not clear from the assumptions of that study whether the accumulation of positive charges required to maintain the transmembrane potential, as shown by this study, would have or should have produced an accumulation of positively charged ANS on the cytosol side of the inner membrane. The equilibrium condition of uniform chemical potentials require that charged species, such as ANS, concentrate near the surfaces of membranes supporting transmembrane potentials, even in the absence of attractive negative surface charges.

Lemasters' measurement using laser scanning confocal microscopy, while

offering improvements in resolution by excluding signal originating outside of the focal plane, and in providing greater depth information, is limited in resolution by the wavelength of light.[9] The range of possible scales z_0, which depends on the dielectric permittivity of the mitochondrial crystae, is under the limit of resolution of such instrumentation.

5 Conclusions

Mitochondria are chemically complex systems whose ATP syntheses utilize a biochemical energetic intermediary in the form of a transmembrane potential induced by a net charge separation across the membrane. Yet the physical relationship between that transmembrane potential and the distribution of the separated charges remains fairly simple even in that environment. However, there is a problem in that the putative charge densities commonly quoted are not of sufficient magnitude to account for the size of the measured transmembrane potentials. This is supported by other considerations of scale in that more H^+ is required simply to populate a mitochondria with more than an occasional ion, a condition for which chemical evidence exists independent of the electrostatic considerations developed here.

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