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Are mitochondria mesoscopic?

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Abstract

Systems whose length scales are small enough to be near the threshold where macroscopic thermodynamic descriptions fail are called mesoscopic. This is particularly appropriate for studies of mitochondrial function since basic and important ideas such as protomotive force, and models finding common applications, such as the Gouy–Chapman theory describing the diffuse ionic charge layer near the inner membrane, depend on the macroscopic thermodynamic notion of chemical potential. This paper shows that the length scales in Gouy–Chapman theory are not consistent with the premises upon which Gouy–Chapman theory was based, a failure typical of mesoscopic systems. Furthermore, while there are good reasons for a protomotive force to be a valid description for a collection of mitochondria, it is a marginal description even for an entire individual mitochondrion for similar reasons. Mitochondria are, therefore, physically mesoscopic systems. © 2001 Elsevier Science B.V. All rights reserved.

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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 [1–3]. 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 cytosol is typically strongly buffered since biological processes require a nearly neutral pH. Most of the H^+ is replaced by other positive ions by this buffering process. The source of the potential gradient is a net accumulation of the positive ions on the cytosol side of the inner membrane, with a concomitant accumulation of negative ions in the matrix.

The chemical potential for H^+, when expressed

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in units of voltage, is usually referred to as 'proto-motive force' and is composed of two parts:

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

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. This is essentially the thermodynamically available energy to drive the protons through ATPase in conditions of constant temperature and pressure. The $\Delta kT \ln n_0$ represents the osmotic energy available due to the concentration gradient across the membrane, and the $e\Delta\psi$ is the energy available due to the potential difference across the membrane.

Thermal equilibrium requires uniformity of the chemical potentials for the charged species throughout the cytosol. If the chemical potential is uniform, then:

$$\vec{\nabla}\mu_{\pm} = \vec{0} = \pm e\vec{\nabla}\psi + kT\vec{\nabla}\ln\rho_{\pm}, \qquad (2)$$

where $\rho_{\pm}$ is the density of positive (negative) ions. This implies that, in thermodynamic equilibrium with solvent, an ionic gradient exerts a force on the ions equivalent to an applied force $\pm e\vec{E} = \mp e\vec{\nabla}\psi + kT\vec{\nabla}\ln\rho$. From this, it also follows that the total thermally available potential energy per particle, including thermodynamic and diffusive effects across a membrane barrier, is the gradient of the chemical potential across the membrane.

Furthermore, the electric field must also satisfy electrostatic conditions

$$\vec{\nabla}\cdot\vec{E} = (\rho_+ - \rho_-)/\varepsilon \qquad (3)$$

In a planar geometry, the combination of a uniform chemical potential in the sol and Gauss' law in a planar geometry is called the Gouy–Chapman model [4–6] in a spherical geometry; this is the Debye–Hückle model [6].

Given the processes mediated by transmembrane potentials that involve protein folding or conformational changes necessary to enzymatic function, ion transport, phosphorylation and other important functions [7], it would be desirable to characterize that electrostatically energized environment in terms of a few simple parameters. Such a simplified environmental specification or parameterization would be a great aid to bioinformatic studies, such as determining the relationship between conditions proximal to electrostatically energized membranes and the evolutionary preference of chemical equivalence substitutions of peptides.

The ions are in thermal equilibrium with their solvent, which acts as a heat bath. While individual ions may pursue individual trajectories, their bulk behavior may be characterized by a macroscopic thermodynamic description. Fluctuations about the macroscopic thermodynamic values reflect the effects of the individual trajectories of the system, which become more important as the system sizes under consideration shrink. Macroscopic description fails when the length scales under consideration are small enough so that those fluctuations due to the details of the individual trajectories dominate the macroscopic parameters. Systems whose sizes are near this transition scale have been characterized as being 'mesoscopic' — not quite large enough to be thermodynamic, and not quite small enough to be simply microscopic. This represents a technological barrier to some semiconductor technologies, opportunities in nanotechnology applications, and has been attracting interest and attention in the physics community.

The literature provides reviews of measurements of $\Delta\psi$ and pH^+, [3,8], including some focused on related issues such as complications of the stoichiometry [9,10] as well as analysis of chemical probes [10–12], including recent measurements with higher spacial resolution achieved with confocal microscopy [13]. Measurements of potentials have also been performed using electrodes [14,15]. Recent analysis, measurements, and reviews of charge distributions in the vicinity of the surface of membranes generally discuss two-component Z–Z electrolytic solutions [7,11,16,17] and employ the Gouy–Chapman [4,5] theory. However, little has been stated about the implication of system scale sizes on the validity of thermodynamic assumptions.

This paper considers the question of whether the environment of the mitochondrial membrane is macroscopic, microscopic or perhaps mesoscopic. More particularly, the question of whether the notion of a continuous charge distribution appropriate to Gouy–Chapman theory can be applied at the concentrations appropriate to mitochondria is considered. It is shown that the predicted screening length is considerably smaller than the characteristic length separating the ions in solution. This implies that the ions are too discrete and sparse to produce a screening effect within a Debye length in the manner suggested by the Gouy–Chapman model. Even if the Gouy–Chapman theory approximates observations, the underlying mechanisms must be more subtle and complex. More importantly, they do not reflect conditions at length scales experienced by enzymes proximal to the membrane.

Section 2 reviews the Gouy–Chapman theory, Section 3 considers the consistency of the Gouy–Chapman theory at these length scales, and Section 4 presents conclusions.

2. Gouy–Chapman theory

This section reviews the Gouy–Chapman theory highlighting those features that connect the Gouy–Chapman theory to common notions such as proticity in application to mitochondria.

The first assumption of the Gouy–Chapman theory is that the chemical potential of charged species is constant in the electrolytic solution. This chemical potential depends on the electrostatic potential ψ and the charge on the free ions $\pm Ze$:

$$\mu_{\pm} = \mu^0_{\pm} + kT\ln\rho_{\pm} \pm Ze\psi \tag{4}$$

The distribution of charges must then satisfy:

$$\rho_{\pm} = \rho^0_{\pm} e^{\mp Ze\psi/kT} \tag{5}$$

Physiological conditions show a cytosol concentration roughly equivalent to 0.155 M NaCl, or 0.31 M sucrose (two times NaCl since sucrose is non-dissociative) for an isotonic solution. The contribution of the fraction of solute that is ionic ranges between 0.1 and 0.15 M, with 0.14 M being reasonably representative. The cytosol is also a strong buffer. The primary mechanism for the transfer of charge out of the mitochondrial matrix is the pumping of H^+. However, almost all of those H^+ will be replaced by other positive ions by the cytosolic buffering action. The solution must remain mildly acidic to maintain function for ATP synthase. Since this discussion focuses primarily on obtaining an order-of-magnitude estimation of length scales, only singly ionized species will be considered.

Furthermore, the free ionic distributions must also act as sources of electric field and electrostatic potential, satisfying:

$$E = -\frac{d\psi}{dz} \tag{6}$$

$$\frac{dE}{dz} = \frac{1}{\varepsilon}(\rho_+ - \rho_-) \tag{7}$$

The boundary conditions require neutrality as $z \to \infty$, so that $\rho \propto d^2\Psi/dz^2 \to 0$, and the electric field $E = -d\Psi/dz \to 0$ so that the charge distributions will not be driven from a neutral equilibrium. More formally,

$$\lim_{z\to\infty} \frac{d^2\Psi}{dz^2} = 0 \tag{8}$$

$$\lim_{z\to\infty} \frac{d\Psi}{dz} = 0 \tag{9}$$

Furthermore, the location where $\psi = 0$ is essentially arbitrary. It may be chosen so that:

$$\lim_{z\to\infty} \Psi = 0 \tag{10}$$

The other boundary that must be satisfied is that at the surface $z \to 0^+$. At the surface, $\psi = \psi_0$ and $E = E(0^+)$.

These equations may be simplified somewhat by identifying:

$$\zeta z_0 = z \tag{11}$$

$$\Psi = \frac{e\psi}{kT} \tag{12}$$

$$\varepsilon = \frac{z_0 e}{kT} E \tag{13}$$

$$r_{\pm} = \frac{z_0^2 e}{\varepsilon kT} \rho_{\pm} \tag{14}$$

$$r_{\pm}^0 = \frac{z_0^2 e}{\varepsilon kT} \rho_{\pm}^0 \tag{15}$$

where z_0 will be defined in terms of the boundary conditions. The differential equations then reduce to:

$$\frac{d\Psi}{d\zeta} = -\mathcal{E} \tag{16}$$

$$\frac{d\mathcal{E}}{d\zeta} = r_+^0 e^{-\Psi} - r_-^0 e^{\Psi} \tag{17}$$

The boundary conditions will be selected so that $\mathcal{E}(0^+) = -1$. From this it follows that:

$$z_0 = \frac{kT}{eE(0+)} \tag{18}$$

where $z_0 > 0$.

The differential equations may be written as:

$$\frac{d^2\Psi}{d\zeta^2} = \frac{1}{2}\frac{d}{d\Psi}\left(\frac{d\Psi}{d\zeta}\right)^2 = -(r_+^0 e^{-\Psi} - r_-^0 e^{\Psi}) \tag{19}$$

As $\zeta \to \infty$, the left hand side goes to zero, and $\Psi = 0$, so that $r_0^+ = r_-^0$. This equation may be integrated to yield:

$$\frac{1}{2}\left(\frac{d\Psi}{d\zeta}\right)^2 = r_+^0 e^{-\Psi} + r_+^0 - e^{\Psi} + C$$

Applying the same boundary conditions as $\zeta \to \infty$ yields $C = -2r_+^0$, so that:

$$\frac{1}{2}\left(\frac{d\Psi}{d\zeta}\right)^2 = r_+^0 e^{-\Psi} - 2r_+^0 + r_+^0 e^{\Psi}$$
$$= r_+^0 (e^{-\Psi/2} - e^{\Psi/2})^2 = 4r_+^0 \sinh^2\frac{\Psi}{2}$$

so that

$$\frac{d\Psi}{d\zeta} = \pm(8r_+^0)^{1/2}\sinh\frac{\Psi}{2}$$

This may be integrated to yield

$$\int\frac{d\Psi}{\sinh\frac{\Psi}{2}} = 2\ln\ \tanh\frac{\Psi}{4} = \pm(8r_+^0)^{1/2}\int d\zeta + C''$$

or

$$\tanh\frac{\Psi}{4} = C' e^{\pm\sqrt{2r_+^0\zeta}}$$

The '−' sign must be selected so that $\Psi \to 0$ as $\zeta \to \infty$. Furthermore, $\Psi(0+) = \Psi_0$, so that

$$\tanh\frac{\Psi}{4} = \tanh\frac{\Psi_0}{4} e^{-\sqrt{2r_+^0\zeta}} \tag{20}$$

Furthermore, at $\zeta = 0^+$, $d\Psi/d\zeta = -\mathcal{E} = 1$, so that

$$\sinh\frac{\Psi_0}{2} = -(8r_+^0)^{-1/2} \tag{21}$$

Thus, given ρ_+^0 and $E(0^+)$, Ψ_0 may be computed, and the corresponding net charge density $\rho = \rho_+^0 \sinh\Psi_0$ at $z = 0^+$ may be determined.

The characteristic screening length s may be defined as $z/s = \sqrt{2r_+^0\zeta}$, so that

$$s = z_0(2r_+^0)^{-1/2} = \sqrt{\frac{\varepsilon_{H_2O}kT}{2e\rho_+^0}} \tag{22}$$

essentially the Debye length from the Debye–Hückle theory of ionic solutions. It is important to note that this length does not depend on $E(0^+)$.

The determination of $E(0^+)$ depends on the membrane itself. The membrane has a dielectric susceptibility ε_m, which is essentially $\kappa_m \varepsilon_0$, where measurements for κ_m are elusive, with suggested values ranging somewhere between 1 and 10. The phospholipid bilayer presents hydrophilic groups to the cytosol. Those groups carry slightly negative charges. The transmembrane proteins also carry charges, some of which may be internal to the membrane, but which may also contribute significantly to a surface charge. Furthermore, the ions in the cytosolic solution may exhibit adsorption. Models for adsorption include the Helmholtz model (simple voltage drop proportional to the surface charge density squared σ^2 as determined from surface tension arguments [6] and the Langmuir [19] adsorption isotherm, which is simply an equilibrium condition between free ions, free surface sites, and the bound surface sites, also accounting for the total number of available surface sites [7]. Inclusion of adsorption into the Guoy–Chapman theory was studied by Stern [18], which cited Langmuir: '...Die Aufgabe, die Anzahl der Ionen pro cm^2 Grenzfläche aus ϕ zu berechnen, ist dann genau die gleiche, wie sie durch die (Langmuirche) Adsorptionstheorie gelöst wird', without specific journal citation. Another simple isotherm is derived from the notion that adsorbed ions are bound to the surface by some simple binding potential, but are otherwise free to move on the surface as a two-dimensional ideal gas; this also provides for a pressure or surface tension contribution to the chemical potential of ions bound to the surface. Even simple measurements of surface charges have been difficult and controversial in mitochondria, not to mention the inherent difficulties of measuring adsorption equilibrium constants in systems such as mitochondria [11].

Given some surface charge density σ, which includes adsorption and bound or intrinsic surface charges, the equation governing the electric field at the boundary is obtained by applying Gauss' law to a flat pillbox with surface area A, giving

$$\oint \varepsilon \vec{E} \cdot \hat{n} da = \left(\varepsilon_{\mathrm{H_2O}} E(0^+) - \varepsilon_m E(0^-)\right) A = \sigma A$$

where $E(0^-) = -V_0/L$, V_0 is the transmembrane potential, and L is the membrane thickness. This yields:

$$E(0^+) = \frac{\sigma}{\varepsilon_{\mathrm{H_2O}}} + \frac{\varepsilon_m}{\varepsilon_{\mathrm{H_2O}}} E(0^-) \tag{23}$$

Ideally, σ would partly depend on the net charge density $\rho(0^+)$, which in turn depends on a through $E(0^+)$.

Robertson's application of the Gouy–Chapman theory together with Langmuir adsorption isotherms [11] was to the question of how significant the contribution of the binding of 1-anilinonapthalene-8-sulfonate (ANS) to putative negative surface charges [20] is, and whether instead the ANS was concentrating in the predominantly negatively charged mitochondrial matrix. Their calculation included singly and doubly charged ions, but only balanced Z:$-Z$ species. They followed an outline similar to that described above, assuming Langmuir adsorption isotherms in equilibrium with the diffuse Gouy–Chapman layer. They performed measurements at different potentials and concentrations by modifying the ionic porosity of the inner membrane, thus obtaining sufficient information to compute the surface adsorption equilibrium constant. They concluded that there were no definitive results on a measure of a negative intrinsic surface charge binding ANS.

3. Is the Gouy–Chapman model consistent with mitochondrial conditions?

This paper considers a simple calculation that should be representative of values typical of mitochondrial conditions for the purposes of estimating the length scales inherent in the theory; the values are not offered as being in any way exact, nor are the conditions represented sufficiently complex to represent the processes in a mitochondrion. In particular, the Gouy–Chapman theory requires consistency between several intrinsic length scales.

The physical constants assumed for this calculation [21], together with parameters descriptive

Table 1
Constants

Constant	Value
$\varepsilon_0[\mathrm{C}^2\mathrm{N}^{-1}\mathrm{m}^{-2}]$	8.85×10^{-12}
ε_m	$\kappa_m\varepsilon_0$
$\varepsilon_{\mathrm{H2O}}$	$\kappa_{\mathrm{H_2O}}\varepsilon_0$
κ_{H2O}	78.5
T[K]	298
$K[\mathrm{JK}^{-1}]$	1.38×10^{-23}
e[C]	1.6×10^{-19}
L[m]	6.6×10^{-9}
V[V]	0.14
ρ_+^0/e[M]	0.14
$\rho_+^0/\mathrm{e}[\mathrm{m}^{-3}]$	8.428×10^{25}
$\rho_+^0[\mathrm{Cm}^{-3}]$	1.3488×10^{7}
$p(\rho_+^0/e)$	0.854
$(\rho_+^0/e)^{-1/3}$[m]	2.2808×10^{-9}
s[m]	8.14×10^{-10}

of the system, such as membrane characteristics [22] and results that hold independent of the field and most membrane characteristics, are given in Table 1. Results obtained from these approximate conditions are listed in Table 2.

First, there is the Debye length measuring the characteristic extinction length of the electrostatic field. These lengths do not depend on any of the characteristics of the system except the temperature and the ionic charge.

The value for s at $T = 298$ K is:

$$s = 0.814 \text{ nm} \tag{24}$$

A test computation under the above conditions with no surface charges or adsorption assumed places the dimensionless potential Ψ between -8.55×10^{-3} and -8.55×10^{-2}, which is less than 1%. This implies that a linear approximation is appropriate in this regime. Net charge density at $z = 0^+$ are seen to be between 1.2×10^{-3} M and 1.2×10^{-2} M, depending on κ_m. This would suggest a characteristic length between unbalanced ionic charge units suggesting a scale of $\rho(0^+/e)^{-1/3}$ ranging between 11.1 and 5.18 nm. This is significantly larger than the screening length s. Since there is no distribution of isolated positive charges, the net charge distribution is rather a net difference between positive and negative ionic distributions, it is reasonable to suggest that perhaps the close cancellation of much larger ρ_+ and ρ_- would effectively permit a nearly continuum net charge distribution. However, the length between ions at 0.14 M is:

$$(\rho_+^0/e)^{-1/3} = 2.28 \text{ nm}. \tag{25}$$

The concentration of ions is not sufficient to support cancellations necessary for the construction of such a small skin depth. The adsorption of positive charges would exacerbate these problems of scale. Negative intrinsic surface charges would reduce the problem somewhat.

At least one requirement for consistency in this context is that $(\rho_+^0)^{-1/3} \ll s$, or

$$\rho_+^0 \gg \left(\frac{2e}{\varepsilon_{\mathrm{H_2O}}kT}\right)^3 \tag{26}$$

Table 2
Predicted mitochondrial H^+ distribution characteristics

Parameter	$\kappa_m = 1$	$\kappa_m = 2$	$\kappa_m = 5$	$\kappa_m = 10$
$E(0^+)[\mathrm{Vm}^{-1}]$	-2.70×10^{5}	-5.40×10^{5}	-1.35×10^{6}	-2.70×10^{6}
z_0[m]	9.51×10^{-8}	4.76×10^{-8}	1.90×10^{-8}	9.51×10^{-9}
r_0	6.83×10^{3}	1.71×10^{3}	2.73×10^{2}	6.83×10^{1}
Ψ	-8.55×10^{-3}	-1.71×10^{-2}	-4.28×10^{-2}	-8.55×10^{-2}
$\rho_0/e[\mathrm{m}^{-3}]$	7.21×10^{23}	1.44×10^{24}	3.61×10^{24}	7.22×10^{24}
ρ_0/e[M]	1.20×10^{-3}	2.40×10^{-3}	5.99×10^{-3}	1.19×10^{-2}
$(\rho_0/e)^{-1/3}$[m]	1.11×10^{-8}	8.85×10^{-9}	6.52×10^{-9}	5.18×10^{-9}
$p(\rho/e)$	9.92	2.62	2.22	1.92

4. Conclusions

When a continuum charge distribution consistent with the maintenance of a uniform chemical potential is assumed as the source for electrostatic fields, the implied self-screening length is smaller than the characteristic distance between the ions in the solution at this concentration of free ions. Even the predicted levels of concentration only shift the uncharged values by less than 1%. This implies that a consistent formulation cannot be constructed based upon a continuum approximation. This places the environment of the inner mitochondrial membrane in the mesoscopic regime. Computations that take into account the discrete character of the charges in the ionic solution are required for an adequate treatment of phenomena at this scale. However, the Gouy–Chapman theory agrees unreasonably well with the results of such simulations, and some arguments have been promoted that explain why it does. The thin diffusion layer of the Gouy–Chapman theory in such a low concentration of counterions suggests an adsorption process much more than it describes a diffuse layer. Even if some of the numerical features do agree fairly well, the large counter ion concentrations assumed in the Gouy–Chapman model is compromised, and the description of the microscopic environment required to predict detailed enzymatic function in that environment fails.

McLaughlin [7] presents an exhaustive review of Gouy–Chapman theory and its applications to biological systems. He reviews the discrete charge problem, but this seems to be associated with discrete charges within or on the lipid membrane. He notes that finite size effects become important only when the ions are larger than the Debye length. He did not discuss the particular problem described here.

The protomotive force is a chemical potential. However, most of the ionic charge that accounts for the transmembrane potential are *not* H^+ ions. The chemiosmotic hypothesis postulates a protomotive force with components that include osmotic and potential difference contributions to the thermally available energy for the function of ATP synthase. The H^+ ions are in solution with the solvent, which acts as a heat bath. Thermal energy is available to the H^+ ions besides just the electrostatic potential gradient. On scales that involve large numbers of mitochondria, this is measured effectively by the osmotic contribution to the chemical potential. The protomotive force is independent of the detailed predictions of the Gouy–Chapman model, and usually would not suffer from the internal inconsistencies of any model of the diffuse layer. However, the concentration corresponding to a $\mathrm{pH} = 6.3$ is $n \sim 5 \times 10^{-7}$ M implying an inter-ion distance of $n^{-1/3} \sim 1.49 \times 10^{-7}$ M, suggesting such osmotic contributions make marginal sense on length scales comparable to single mitochondria, and much less sense on a fold of the crystae. There are only a small number of H^+ ions available in any single mitochondrion at any one time. Protomotive force does make much more sense if many mitochondria are considered. Local potentials are going to be complicated, reflecting the fields of individual positive ions rather than the simple smoothness of a continuum charge distribution. This is smoothed out in consideration of all the crystae in a number of mitochondria. This implies that local protomotive force should be considered with care, showing many of the more difficult characteristics of mesoscopic systems.

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