Fluctuations of the proton-electromotive force across the inner mitochondrial membrane

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The intermembrane mitochondrial space (IMMS) is delimited by the inner and outer mitochondrial membranes and defines a region of molecular dimension where fluctuations of the number of free protons and of transmembrane voltage can give rise to fluctuations in the proton-electromotive force E_{PMF} across the inner mitochondrial membrane (IMM). We have applied the fluctuation-dissipation theorem to an electrical equivalent circuit consisting of a resistor R_m in parallel with a capacitor C_m representing the passive electrical properties of the IMM, in series with another capacitor C_b representing the proton-buffering power of the IMMS fluid. An access resistance R_a was defined as a link between the capacitor C_b and the membrane. Average E_{PMF} fluctuations across the IMM were calculated for different assumptions concerning the intermembrane space dimensions. The calculated average E_{PMF} fluctuations were in the vicinity of 100 mV for relaxation times in the few-microseconds range. The corresponding fluctuational protonic free energy is about 10 kJ/mole, which is comparable to the binding energy for protons in different transporters. This suggests that fluctuations in E_{PMF} can be of relevance in the universe of forces influencing the molecular machinery embedded in the IMM. [S1063-651X(97)01205-1]

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I. INTRODUCTION

The synthesis of ATP in eukariotic cells occurs at the expense of proton flow driven by the proton electromotive force (E_{PMF}) across ATP synthase molecules inserted in the inner mitochondrial membrane (IMM). Proton gradients as high as 1 pH can occur at that location [1] and superposition with electrical potential difference can lead to E_{PMF}'s attaining the 200-mV mark.

Since the intermembrane space in most of its trajectory has a width of only about 10–20 nanometers, this dimension limits a region of molecular proportions where fluctuations in thermodynamic parameters may become an important part of the forces acting upon the molecular machines inserted alongside the inner mitochondrial membrane.

This paper analyzes some consequences of the particular geometry of the intermembrane space (IMS) in mitochondria (IMMS), which may result in relatively important fluctuations of proton-electromotive force across the IMM.

II. THEORY

The free-energy change ΔG for the creation of an electrochemical gradient by an ion pump is [1] (the SI system of units is employed throughout)

$$\Delta G = RT \ln \frac{c_2}{c_1} + zF\Delta\Psi, \tag{1}$$

where $c_2 c_1^{-1}$ is the concentration ratio for the ion that moves, z is the ion valence, F is Faraday's constant, R is the gas constant, T is the absolute temperature, and $\Delta\Psi$ is the transmembrane difference in electrical potential measured in volts. The proton-electromotive force is defined by

$$E_{PMF} = 2.3\frac{RT}{F}\Delta p\mathrm{H} + \Delta\Psi. \tag{2}$$

When $\Delta G = 0$ (zero chemical driving force, $E_{PMF} = 0$), Eq. (3) can be used to relate the variations of pH across the membrane with voltage changes

$$\Delta p\mathrm{H} = -\frac{F\Delta\Psi}{2.3RT} = -\frac{e\Delta\Psi}{2.3kT}, \tag{3}$$

where e is the electronic charge and k the Boltzmann constant.

In order to determine the fluctuations of the E_{PMF} across the IMM, we need to know the susceptibility $\alpha(\omega)$ of the system comprised of the following elements (see Fig. 1): (1) the inner mitochondrial membrane with its associated resistance and capacitance; (2) the proton buffer compartment associated with the intermembrane fluid; (3) the access resistance between the first two elements.

In doing the above associations we can model the system as a set of electrical resistors and capacitors. The IMM and its surrounding solutions are represented by the *membrane capacitor* C_m. The conductive pathways across the IMM, which include the pump channel and other leaks (including decouplers), are collectively represented by the term R_m. The buffering capacity of the intermembrane fluid is represented by an electrical equivalent (calculated below) defined as $C_{buffer} = C_b$. This compartment is electrically connected to the IMM by an access resistance R_a.

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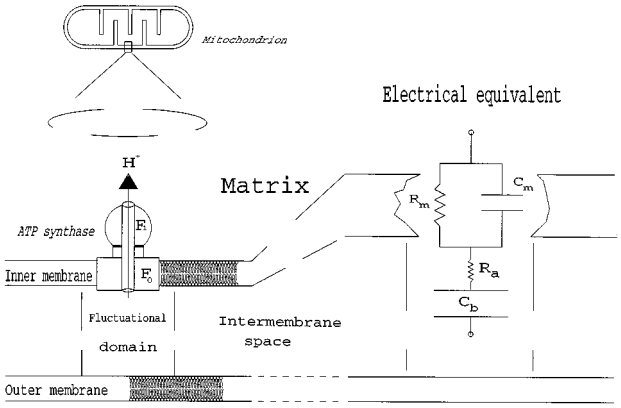

FIG. 1. Diagram showing the mitochondrial membrane and electrical equivalent.

In this way, the relation between the Fourier components of the fluctuational charge q_ω and the corresponding components of the proton-electromotive force at that frequency $(E_{PMF})_\omega$ is (see, for instance, Procopio and Fornés [2])

$$q_\omega = \alpha(\omega)(E_{PMF})_\omega . \tag{4}$$

The impedances of the membrane Z_m and of the buffer system Z_b can be written respectively as

$$\frac{1}{Z_m} = \frac{1}{R_m} + i\omega C_m , \quad \frac{1}{Z_b} = R_a + \frac{1}{i\omega C_b}, \tag{5}$$

where i is the imaginary unit. The total impedance Z_T is given by $Z_T = Z_m + Z_b$. From the relation between the suceptibility and the impedance $\alpha(\omega) = i/(\omega Z_\omega)$, we get for the real $\alpha'(\omega)$ and imaginary $\alpha''(\omega)$ parts of the suceptibility

$$\alpha'(\omega) = -\frac{(\omega R_m \tau_m)/[1+\omega\tau_m)^2] + R_a/(\omega\tau_b)}{\omega D(\omega)}, \tag{6}$$

$$\alpha''(\omega) = -\frac{R_m/[1+(\omega\tau_m)^2] + R_a}{\omega D(\omega)}, \tag{7}$$

where $D(\omega)$ is given by

$$D(\omega) = \left[\frac{\omega R_m \tau_m}{1+(\omega\tau_m)^2} + \frac{R_a}{\omega\tau_b}\right]^2 + \left[\frac{R_m}{1+(\omega\tau_m)^2} + R_a\right]^2, \tag{8}$$

and where $\tau_m = R_m C_m$ and $\tau_b = R_a C_b$ are the corresponding relaxation times of both systems.

Then the corresponding spectral density of the mean square of the fluctuational proton-electromotive force $[(E_{PMF})^2]_\omega$ will be given by [2]

$$[(E_{PMF})^2]_\omega = \frac{\alpha''(\omega)}{|\alpha(\omega)|^2}\frac{2kT}{\omega}, \tag{9}$$

where $|\alpha(\omega)|^2 = [\alpha'(\omega)]^2 + [\alpha''(\omega)]^2$. The mean square of the fluctuating proton-electromotive force acting across an IMM patch is given by the integral

$$\langle (E_{PMF})^2 \rangle = \frac{1}{\pi}\int_0^\infty [(E_{PMF})^2]_\omega d\omega. \tag{10}$$

III. PARAMETER DEFINITIONS

In order to estimate the IMM patch capacitance, we consider an idealized mitochondrion with an intermembrane space about 15–20 nm wide. This minimum dimension limits a cube with 15–20 nm side that defines a fluctuational domain (see Fig. 1).

The intermembrane space (the space between the two mitochondrial membranes) can then be partitioned into a great number of parallel volumes that are considered to function independently, i.e., are uncorrelated. The IMM patch lining each of these elementary volumes then has an area of 225–400 nm^2. The patch electrical capacity is given by

$$C_m = \epsilon\epsilon_0 \frac{A}{d}, \tag{11}$$

where A is the area of the small IMM patch lining each elementary volume, ϵ and ϵ_0 are, respectively, the relative dielectric constant of the membrane ($\epsilon = 2$) and permittivity of vacuum ($\epsilon_0 = 8.85\times10^{-12}$ F m^{-1}), and d is the IMM membrane thickness.

IV. CALCULATION OF BUFFER-EQUIVALENT ELECTRICAL CAPACITANCE

The change of electrical charge ΔQ associated with a given change ΔpH of the pH of an elementary volume V of the intermembrane fluid is given by

$$\Delta Q_b = FV\Delta[H^+] = FV\beta\Delta p\mathrm{H}, \tag{12}$$

where β is the specific buffering capacitance of the intermembrane fluid equal to 10 mM of acid added per pH unit change, $\beta = \Delta[H^+]/\Delta p\mathrm{H}$ (see Läuger [3]). This charge, stored in buffer capacitance, corresponds to a voltage change given by

$$\Delta\Psi_b = \frac{2.3RT}{F}\Delta p\mathrm{H}. \tag{13}$$

The electrical equivalent of the buffer capacitance will then be

$$C_b = \frac{\Delta Q_b}{\Delta\Psi_b} = \frac{\beta F^2 V}{2.3RT}. \tag{14}$$

V. CALCULATION OF IMM ELECTRICAL RESISTANCE R_M

We assume a density of ATP synthase molecules such that there is an average of 1 pumping unit facing each cubic fluctuational domain whose lateral area is about 400 nm^2. The single-channel conductance of the ATP synthase is taken as 1 pSiemens at approximately pH 7.4 (Lauger [3], Lill, Altheff, and Junge [4]), which corresponds to a resistance $R_m = 1\times10^{12}\ \Omega$.

VI. RELAXATION TIMES OF THE ELECTRICAL AND BUFFER RESERVOIRS

Coupling between the buffer and the membrane systems is expected to be of importance when the characteristic re-

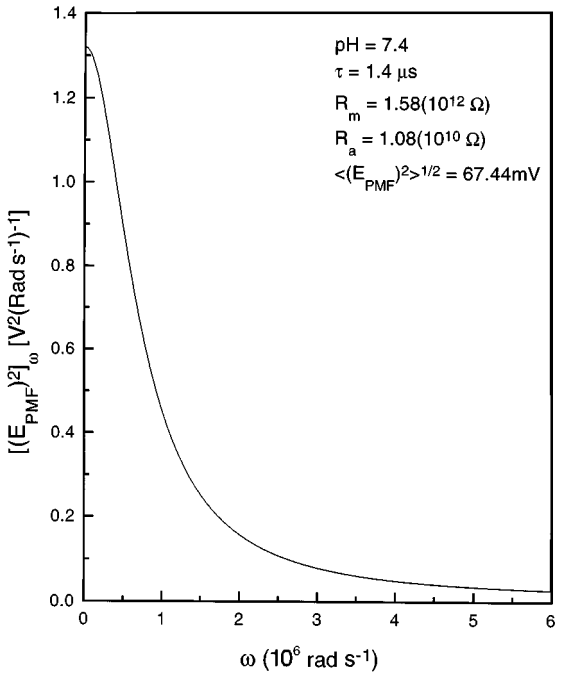

FIG. 2. Spectral density of the mean square of the fluctuational E_{PMF} (Eq. 9).

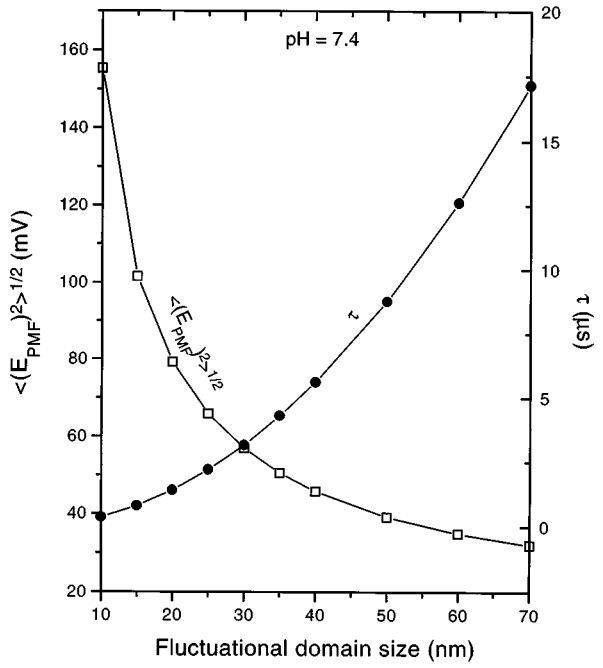

FIG. 3. Representation of $\langle (E_{PMF})^2 \rangle^{1/2}$ and the associated relaxation times (τ) as a function of the possible sizes of fluctuational domains.

laxation times (as defined by their corresponding RC) of both systems are of the same order.

We use this condition to calculate the access resistance R_a of the electrical link between the IMM and the buffer compartment, namely, $\tau = \tau_m = \tau_b$, assuming that proton movement between the membrane and buffer compartments is fast relative to the proton translocation across the IMM (see Sec. VII). From this condition we obtain

$$R_a = \frac{\tau}{C_b}. \tag{15}$$

VII. RESULTS AND DISCUSSION

Figures 2 and 3 summarize our results. The coupling condition of the relaxation times of the membrane and buffer systems [Eq. (17)] makes the ''spectral density'' function [Eq. (11)] decrease asymptotically with increasing radial frequency (see Fig. 2). This creates a convergence condition for the integral of Eq. (13), allowing for the determination of the mean square E_{PMF} fluctuation for a range of fluctuational domain sizes (Fig. 3).

Figure 3 spans a relatively wide range of possible sizes of fluctuational domains and displays the corresponding mean E_{PMF} fluctuation and associated relaxation times. The factors limiting the size of a fluctuational domain were defined by its physical barriers (delimiting membranes, etc.) and the lack of correlation with neighboring domains (distance factor). In the present case, the physical limits are defined by the two mitochondrial membranes, limiting the extension of the intermembrane space. We chose this as a maximum size for a fluctuational domain, thus defining the value of 20–30 nm as an upper size limit for a fluctuational domain, with 10 nm being a definite size possibility in many types of mitochondria. Taking 15 nm as a ''typical'' width for the IMMS, Fig. 3 indicates a mean E_{PMF} fluctuation of 100 mV at pH 7.4 with a corresponding relaxation time of 1 μs. At another extreme, for a fluctuational domain size of 70 nm we obtain a mean E_{PMF} fluctuation of about 40 mV with a corresponding relaxation time of 18 μs.

Both the amplitude of the mean E_{PMF} fluctuation and its characteristic relaxation time are relevant in any attempt at estimating the effects of a fluctuating E_{PMF} on the molecular machinery resident in the IMM.

In order to be of relevance, such fluctuational force is required to have a minimum amplitude and duration that are to be compared with the dynamic characteristics of the specific system upon which it acts, in this case protons interacting with ATP synthase molecules. We shall consider two main effects of the fluctuating field upon the proton dynamics in the above system. The first effect concerns free-proton translocation and the second is related to proton association/dissociation with binding sites along the transporter molecule.

The rate of proton translocation associated with ATP synthesis is about 1200 s^{-1}, giving a translocation time of 8×10^{-4} per proton. Assuming that proton translocation across the F_1 sector in either direction is a three-step process (Stein and Läuger [5]), this gives 2.6×10^{-4} s as the mean time associated with each step.

Proton translocation across the F_0 sector is a substantially faster process with typical turnover time of 6×10^5 protons/s^{-1} per channel (for a conductance of 1 pSiemens and a driving force of 100 mV). This defines a transit time for protons across the channel/pump synthase molecule on the order of $1–2\times10^{-6}$ s (see Läuger [3] and Lill, Althoff, and Junge [4]).

Since complete proton translocation across the transporter involves many protonation-deprotonation reactions with spe-

cific sites alongside the channel region, it is expected that the individual steps last only a fraction of the total translocation time, i.e., a fraction of 1 μs. This is to be contrasted with the dwell time for protons in sites, estimated by Kasianowicz and Bezrukov [6] as ranging around the 100 μs mark.

The binding and debinding proton kinetic constants can, in principle, be modified by fluctuational electrochemical forces in the few-μs characteristic times, provided the fluctuating energy has both an amplitude and characteristic time on the same order as the energies involved in proton binding to and debinding from sites.

The product of the fluctuational E_{PMF} by the proton charge gives the fluctuation in proton energy. Taking a fluctuational E_{PMF} of about 100 mV and a proton charge equal to 1.6×10^{-19} C, a fluctuational energy of about 10 kJoule/mole^{-1} is obtained. This is to be compared with the energy barrier for a water pore formation in a bilayer which is about 100 kJoule/mole^{-1} (Marrink, Jahnig, and Berendsen [7]) and 63 kJoule/mole^{-1} for proton dissociation from water (Deamer and Nichols [8]). In biological systems Woodhull [9] finds a pK_a of 5.3 for the proton binding into nerve Na^+ channels that gives $\Delta G = 30$ kJoule/mole^{-1}. More recent studies of Kasianowicz and Bezrukov [6] in the α- toxin channel point to an effective pK_a of 5.5 for proton binding with resulting $\Delta G = 31.5$ kJoule/mole^{-1}. These values of binding energy compare with the estimated fluctuations in proton energy due to the fluctuating E_{PMF}. Therefore, the fluctuational proton energy is of the same order as its binding energy which implicates the above-described fluctuations as influencing proton binding and debinding. Concerning the times involved, Gutman *et al.* [10] have reported protonation and deprotonation times of about 2–3 μs in IMM preparation, which is of the same order as the relaxation time of the E_{PMF} fluctuations we described above.

The access resistance best estimated to match the common relaxation time of the membrane and buffer compartments was found to be $9\times10^9\ \Omega$, which is about 100 times smaller than the proton channel resistance. This value is high as compared with proton resistance of an aqueous pathway having equivalent geometry. We took as an assumption that the limiting factor in proton translocation was the channel-crossing step. In effect, in 1996 DeCoursey and Cherny [11] concluded that H^+ diffusion is not a rate-limiting step in the overall proton translocation across channels.

Additional reason for adjusting the access resistance value is that the mechanisms of proton translocation near or at biological interface are far from being adequately understood. Values of reported, calculated, or predicted proton conductance vary considerably (Nagle and Morowitz [12], Heberle *et al.* [13]). The general consensus, however, is that proton translocation across microdomains is significantly faster compared to bulk water. Since proton conductivities on or near surfaces are subject to great controversy (see Kasianowicz and Bezrukov [6]), it is reasonable to make the access resistance an adjustable parameter, rather than estimate its value from the proton mobility in bulk water and geometric parameters.

Considering that typical time-averaged E_{PMF}'s across IMM are about 140 mV (Läuger [3]), the values of mean E_{PMF} fluctuation reported here can be considered relevant to the proton dynamics in the μs time scale. The effects of this E_{PMF} fluctuation should be interpreted along the following lines

(a) Coupling of E_{PMF} fluctuations with pump-synthase conformational states, different pump states, and their corresponding dwell times (Stein and Läuger [5]).

(b) Coupling of the fluctuations with protonation and deprotonation of sites: part of the influence of the fluctuating E_{PMF} could be felt in the protonation-deprotonation dynamics and part involved in the modulation of the channel conductance for other ions (see Kasianowicz and Bezrukov [6]).

The above-calculated fluctuations of E_{PMF} across the IMM should be interpreted in connection with the molecular machines under their influence. Two general assumptions about the pump-synthase reacting time serve as the basis for our analysis.

(1) The assumption of a ''slow'' pump mechanism translate into a system reacting to only time-averaged E_{PMF}, calculated from the known values of pH and voltage across the IMM.

(2) If the reacting time of the synthase machine is ''fast'' (say, in the 10-μs range), then the fluctuational changes of E_{PMF} may interfere substantially with its function.

We conclude that the 100-mV fluctuations in E_{PMF} having a characteristic time of 1 μs may be relevant for the processes of proton translocation inside the F_0 sector of the ATP-ase. Lesser amplitude E_{PMF} fluctuations with correspondingly longer relaxation times might influence conformational changes in the ATP-ase molecule.

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