

Membrane Remodeling and Diffusion of Cytochrome C from a Geometrically Idealized Mitochondrial Crista

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Abstract

In healthy eukaryotic cells, mitochondria have an outer membrane that surrounds a complex inner membrane structure [7,8]. Cytochrome c is found in the space between the two membranes, the inter-membrane space, where it takes part in the electron transport process. Mitochondria interact with the cell in the process of apoptosis (programmed cell death). During apoptosis cytochrome c is released from the mitochondria into the cytoplasm. It is known that essentially all (more than 90%) of the cytochrome c inside of a mitochondrion is released to the cytosol in one to two minutes. The mechanism by which this release occurs is the subject of this paper.

Structural transformations in the inner mitochondrial membrane have been observed during apoptosis [6]. We consider the hypothesis that such shape restructuring is required for the timely release of cytochrome c during apoptosis. The extent to which shape restructuring of the inner mitochondrial membrane could affect the timing of this release is the mathematical question that is examined. The quantitative model presented here simulates the diffusion of cytochrome c from within the inter-membrane space. The adsorption and chemisorption of cytochrome c onto the inner membrane and the tendency of cytochrome c to bind with other species in solution complicate the problem. These complications effectively move the cytochrome c into remote pools. While many of the rate constants between remote pools are presently unknown, this model considers the rate constants between cytochrome c and complex III and complex IV and their effect upon cytochrome c release and the need for membrane remodeling to produce diffusion times consistent with observed experimental data.

Keywords

Diffusion, apoptosis, cytochrome c release, computer modeling.

Introduction

The mitochondrion is the center of aerobic respiration in eukaryotic cells. This organelle contains two protein/lipid bilayer membranes, an outer membrane that envelops the organelle and a protein rich inner membrane that is extensively folded (See Figure 1).

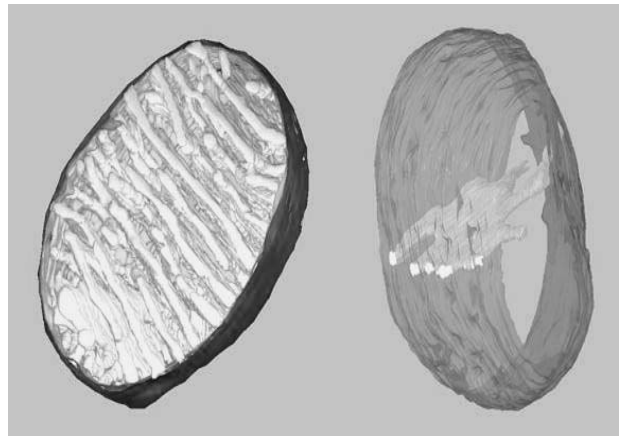


Figure 1 – Left: Tomogram of mitochondrion with cristae in interior region. Right: One crista exhibiting multiple crista junction connections to the inner boundary membrane [7].

The folded regions called cristae connect with the inner boundary membrane at sites known as cristae junctions. These cristae constitute the primary site for oxidative phosphorylation, catalyzed by the electron transport chain [10]. As a part of the electron transport chain the small protein, cytochrome c, transfers electrons from complex III to complex IV. As such, all of a healthy cell's cytochrome c molecules are found within the mitochondria. Although cytochrome c is known to bind with other molecules that are found in the intermembrane space, the scope of this paper is restricted to the interactions with complex III and complex IV.

Cytochrome c is also involved in another critical cellular process known as apoptosis, or programmed cell death. Apoptosis is the physiological process by which damaged or unneeded cells are eliminated by an organism. Two pathways are known by which this process is initiated. The intrinsic apoptotic pathway is one in which mechanisms within the cell trigger the mitochondria to release their cytochrome c and initiate apoptosis. The extrinsic pathway involves a trigger from outside of the cell to cause a cascade of reactions that prompt the mitochondria to release their cytochrome c in apoptosis. Since both of these pathways involve the release of cytochrome c into the cytosol, the dynamics of that release are of particular interest. Observations made of the apoptotic process have led to the development of several theories relating to the structural and chemical changes necessary to release cytochrome c from mitochondria. Three of these theories are shown in Figure 2.

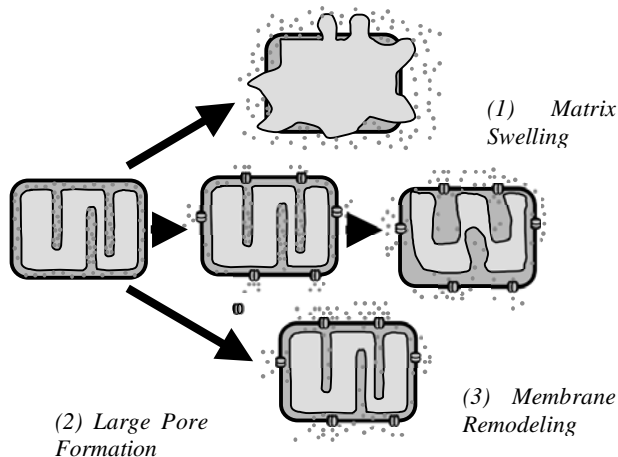


Figure 2 – Three possible mechanisms for the release of cytochrome c from a mitochondrion.

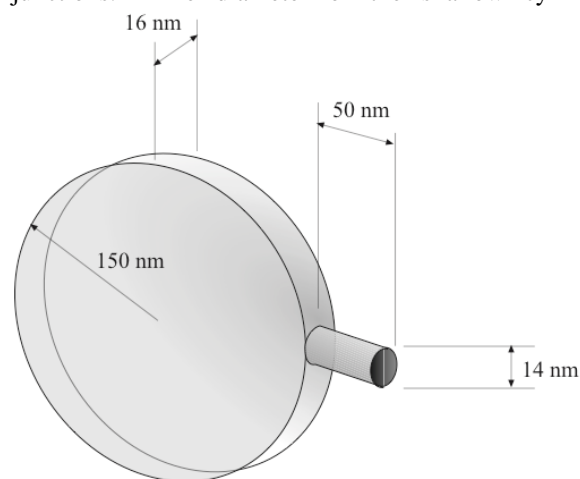
The first of these involves the inner membrane of the mitochondrion undergoing a permeability transition that then leads to the matrix swelling to a point where

the inner membrane ruptures the outer membrane. This rupturing then allows the cytochrome c to exit into the cytosol. While matrix swelling is observed in some experiments, in many cases, cytochrome c has usually left the mitochondrion prior to the swelling and rupturing, therefore the swelling is not likely a necessary factor in cytochrome c release. The second theory described involves the formation of large pores in the outer membrane to allow the release of cytochrome c. The third theory, and the one of interest to this paper involves the second theory followed by a permeability transition in the inner membrane coupled with the remodeling of the inner membrane, in particular, increasing the diameter of the cristae junctions.

The goal of this paper is to determine whether or not membrane remodeling is necessary for cytochrome c to diffuse out of the intra-cristal space into the intermembrane space fast enough to explain its release into the cytosol within a one to two minute time span as observed experimentally [9].

Model

To simplify the modeling of the diffusion problem, a typical crista was geometrically simplified and represented as a shallow closed cylinder with a cylindrical tube extending from its edge (see Figure 3). The diameter for the crista junction was chosen using experimental data that shows an average exterior junction diameter of 24 – 28 nm [5]. When we subtract the thickness of the lipid bilayers, we are left with an average interior diameter of roughly 14 nm for the cristae junctions. The diameter of the shallow cylinder



* All measurements are to the interior wall of the lipid bilayer.

Figure 3 – Geometrically Simplified Mitochondrial Crista and Junction

that represents the lamellar cristae was chosen to be 150 nm, with a corresponding thickness of 16 nm. The tu-

bular section running from the crista junction to the body of the crista was set to 50 nm with the same diameter as the crista junction. With these parameters established, the crista junction area, the volume of the crista and the intra-cristal space surface were calculated to be $1.5394 \times 10^{-16} \text{ m}^2$, $7.698 \times 10^{-18} \text{ L}$, and $1.56451 \times 10^{-13} \text{ m}^2$ respectively.

Initial values for the particle concentrations were derived from data found in [2]. The free cytochrome c concentration was given as $[c_0]=700 \text{ }\mu\text{M}$, which converts to $[c_0]=7.0 \times 10^{-4} \text{ M}$. Using ratios in [3], the concentrations of free complex III and free complex IV were computed to be $[a_3]=1.148 \times 10^{-8} \text{ M}$ and $[a_4]=2.583 \times 10^{-8} \text{ M}$. The results reported in [3] show that, at physiological ionic strength the fraction of bound cytochrome c is 5% of the total cytochrome c present. This is the bound fraction we assume in our calculations. This yields an initial value for cytochrome c bound with complex III to be $[b_3]=5.74 \times 10^{-10} \text{ M}$ and cytochrome c bound with complex IV to be $[b_4]=1.292 \times 10^{-9} \text{ M}$. We use the diffusion constant of cytochrome c as given in [2] to be $1.0 \times 10^{-6} \text{ (cm)}^2\text{/second}$, which converts to $1.0 \times 10^{-10} \text{ m}^2\text{/second}$.

Two models were constructed to simulate the diffusion of cytochrome c from the intra-cristal space. The first, a simple diffusion model, neglects any remote pools of cytochrome c resulting from binding to redox partners. The diffusion of cytochrome c in this model is given by the equation:

$$\frac{d[c]}{dt} = \frac{-\gamma A[c]}{\ell V} \quad (1)$$

where

$[c]$ is the concentration of free cytochrome c, Moles/liter;

γ is the diffusion constant of cytochrome c, $(\text{meter}^2)\text{/second}$;

A is the area of the crista junction opening, nm^2

ℓ is the length of the tube connecting the crista with the crista junction, nm; and

V is the volume of the crista, liter.

The solution to (1) gives us the equation for the simple diffusion of cytochrome c from the intra-cristal space.

$$[c(t)] = c_0 e^{\frac{-\gamma A}{\ell V} t} \quad (2)$$

The second model adds the chemical interaction of cytochrome c with its redox partners, complex III and complex IV, and is characterized by a system of five coupled differential equations.

$$\begin{aligned} \frac{d[c]}{dt} = & -k_3[c][a_3] - k_4[c][a_4] + k_{-3}[b_3] \\ & + k_{-4}[b_4] - \frac{-\gamma A[c]}{\ell V} \end{aligned} \quad (3)$$

$$\frac{d[a_3]}{dt} = -k_3[c][a_3] + k_{-3}[b_3] \quad (4)$$

$$\frac{d[a_4]}{dt} = -k_4[c][a_4] + k_{-4}[b_4] \quad (5)$$

$$\frac{d[b_3]}{dt} = k_3[c][a_3] - k_{-3}[b_3] \quad (6)$$

$$\frac{d[b_4]}{dt} = k_4[c][a_4] - k_{-4}[b_4] \quad (7)$$

where

$[c]$ = free cytochrome c concentration, Moles/liter;

$[a_3]$ = complex III concentration, Moles/liter;

$[a_4]$ = complex IV concentration, Moles/liter;

$[b_3]$ = concentration of cytochrome c bound to complex III, Moles/liter(meter^2);

$[b_4]$ = concentration of cytochrome c bound to complex IV, Moles/liter(meter^2);

Results and Discussion

Using (2) in the simple diffusion model, we fixed the parameter values while varying the length of the tube, ℓ , from our initial value of 50 nm down to 10 nm. Since our interest was in whether or not the cytochrome c will diffuse out of the intra-cristal space in one to two minutes, t was run from 0 to 120 seconds. The results of this exploration are found in Figure 4. The next step in the simple diffusion model was to hold all of the parameters fixed while varying A , the area of the crista junction while holding the external concentration of cytochrome c at ~ 0 , as the volume of the cytosol into which it diffuses is very large compared to the volume of the crista. The diameter of the junction was varied from 14 nm, our default measurement, to 59 nm. These values have a corresponding area A of 154 (nm)^2 and 380 (nm)^2 respectively.

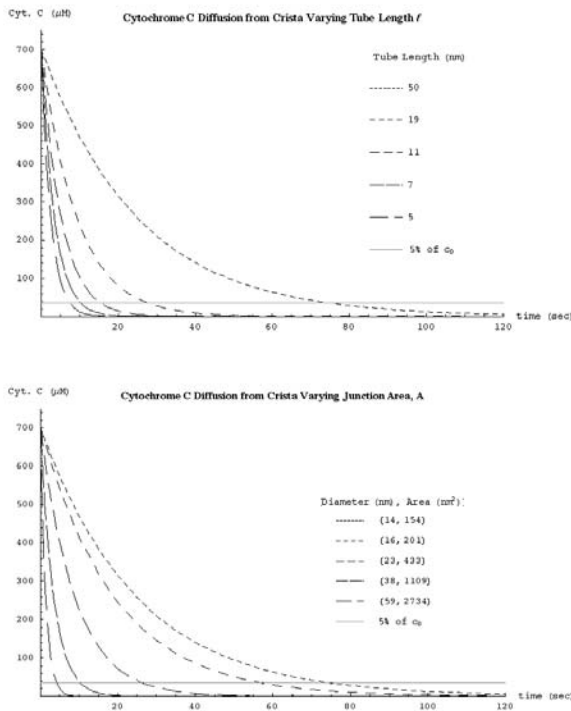


Figure 4 – Simple Diffusion Results. Top: The length of the tube was varied from its initial condition to progressively shorter lengths. Bottom: The diameter of the crista junction was remodeled, starting at the given initial conditions and increased in diameter up to a maximum of 59 nm.

The diffusion of free cytochrome c for several values of the junction area is shown in Figure 4. In this model, diffusion of 95% of the cytochrome c from the crista occurred in under 80 seconds, with 99% diffusing out within 120 seconds. This rate of diffusion was accomplished without the remodeling of either the tube length or the crista diameter. When the remodeling is applied, the rates for diffusion of 95% of the cytochrome c is seen in < 60 seconds.

Our second model, uses two association rate constants, k_3 and k_4 , and two dissociation rate constants, k_{-3} and k_{-4} . The starting equilibrium conditions are obtained by setting equations (3 – 7) to zero. Substituting the reported concentrations of the free and bound species gives us two constraints on the values of these rate constants

$$\frac{k_3}{k_{-3}} = \frac{k_4}{k_{-4}} = 1428.6$$

The results of dynamical simulations with the model were calculated for a grid of possible values of the rate constants ranging between 10^{-8} and 10^6 .

Numerical solutions to the system of equations (3 – 7) were computed holding the junction and tube parameters fixed at the initial values, the solutions to these are found in Figure 5. These solutions were then compared with the simple diffusion model corresponding to the initial tube length and junction diameter. As seen in the simple diffusion model, the cytochrome c is able to diffuse from the intra-cristal space in under two minutes without any remodeling of the tube length or the crista junction.

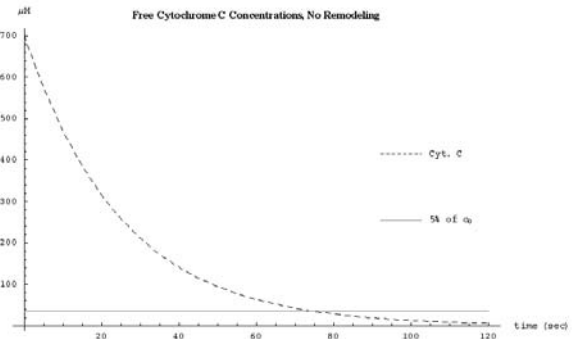


Figure 5 – Results of Diffusion with Chemical Interaction between cytochrome c and complex III and complex IV.

Conclusions

The goal of this project was to determine if remodeling of the mitochondrial crista was necessary for cytochrome c to diffuse out of the intra-cristal space into the inter-membrane space in one to two minutes in keeping with experimental results [9]. We proceeded by constructing two diffusion models based upon a geometrically simplified representation of the crista and crista junction. We then simulated both simple diffusion of cytochrome c and diffusion of cytochrome c while reacting with complex III and IV. In both models, the time it took for 95% of the cytochrome c in the crista to diffuse out into the inter-membrane space was 80 seconds which is consistent with experimental results. This rate of diffusion was accomplished without any membrane remodeling leading to the conclusion that remodeling of the membrane is not a prerequisite to cytochrome c release during apoptosis.

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