Hypothesis

POSSIBLE ROLE OF THE MITOCHONDRIAL OUTER MEMBRANE AS AN ONCOTIC REGULATOR OF MITOCHONDRIAL VOLUME

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1. Introduction

This paper discusses the permeability and osmotic properties of mitochondria in relation to ultrastructural configuration. An oncotic pressure hypothesis is presented which proposes that an osmotic pressure gradient exists across the outer membrane that is equal to the oncotic pressure difference between the outer compartment and the extramitochondrial space. This oncotic pressure gradient is minimal under de-energized conditions when mitochondria assume a condensed configuration. The gradient increases several fold following energization as mitochondria transform to an orthodox configuration. It is concluded that mitochondria become hyperosmotic to their environment under energized conditions with the osmotic gradient borne across the outer membrane. Energy-linked changes in the relative volumes of the inner and outer compartments may therefore be the result of changes in the magnitude of this osmotic gradient. Preliminary data supporting this hypothesis are presented.

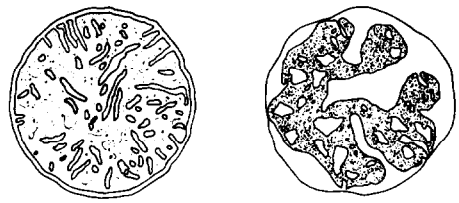
2. Experimental

Rat liver mitochondria were isolated in 0.25 M sucrose [1]. Hypotonically-shocked mitochondria were prepared by dilution of the stock mitochondrial suspension into 45 mM sucrose at 0°C. Intact and shocked mitochondria were resuspended in varying concentrations of sucrose to final mitochondrial protein conc. 0.3 mg/ml [2] and A_{540} immediately measured. Mitochondria were fixed for electron microscopy by centrifugation through 3% glutaraldehyde, 100 mM NaP_i buffer, pH 7.4, 0°C. Mitochondria swollen in 100 mM sucrose were fixed in an identical fashion except that 1.2% glutaraldehyde, 40 mM NaP_i buffer, pH 7.4, was used as the fixative. The pellets were then postosmicated, embedded in epon, thin sectioned, stained with uranyl acetate and lead citrate, and examined in a JEOL 100B electron microscope.

3. Results and discussion

The familiar essentials of mitochondrial ultrastructure are depicted schematically in fig.1. The space between the inner and outer membranes is the outer compartment or intermembrane space. The space within the inner membrane is the inner compartment or matrix space. Two ultrastructural configurations are depicted in fig.1 – orthodox and condensed – as described [3]. In the orthodox configuration, the inner compartment is large and the outer compartment is small. In the condensed configuration, the inner and outer compartments have approx. equal volumes [4].

The permeability properties of the mitochondrial membranes are well established [5]. Unless a specific transporter system is present, the inner membrane is impermeable to most inorganic ions, including protons, and lipid insoluble molecules greater in size than glycerol. The outer membrane, however, is highly permeable. It is impermeable only to molecules of approx. mol. wt $> 10\,000$.



Configuration	ORTHODOX	CONDENSED
Associated metabolic state	Energized	De-energized
Outer compartment oncotic pressure	High	Low
Osmotic pressure gradient	High	Low

Fig.1. Schematic representation of the condensed and orthodox configurations of mitochondria. The relation of mitochondrial configuration to metabolic state, outer compartment oncotic pressure, and osmotic pressure differential across the outer membrane is illustrated.

The inner membrane is extensively convoluted and can unfold to accommodate volume changes of the inner compartment. As a result the inner compartment acts as a perfect osmometer by swelling or shrinking in response to any osmotic pressure gradient [6,7]. By this mechanism, presumably, the osmotic pressures of the inner and outer compartments are identical. Since the outer membrane is impermeable to particles $\geqslant$ 10,000 mol. wt, only this size particle can exert an effective osmotic gradient across the outer membrane. Outer compartment protein concentration and oncotic pressure (i.e., osmotic pressure of solutes of high molecular weight) are estimated in table 1. Oncotic pressure is based on the molecular weight of adenylate kinase mol. wt 21 500), the major identified outer compartment protein [5].

Orthodox mitochondria have an outer compartment volume that is roughly 1/7th that of condensed mitochondria as estimated from electron micrographs.

Table 1

Configurational state	Protein (mg/ml)	Oncotic pressure (protein mol. wt 21 500 = adenylate kinase)
Condensed	60 [4]	3 mOs
Orthodox	420	20 mOs

Therefore, the protein concentration and oncotic pressure of the outer compartment are 7-fold greater in orthodox mitochondria provided there is no aggregation of protein molecules. The net osmotic pressure across the outer membrane is the difference between outer compartment oncotic pressure and oncotic pressure of the suspending medium. Thus, the osmotic pressure across the outer membrane can be as high as 20 mOs under in vitro conditions when suspending medium oncotic pressure is negligible.

Rat liver mitochondria was first noted to transform from an orthodox in situ configuration to a condensed in vitro form during isolation in sucrose solutions [8]. This configurational transformation could be prevented by addition of a high molecular weight solute – polyvinylpyrrolidone – to the isolation medium. These observations have been confirmed and extended [9–11] where high molecular weight dextran, polyvinylpyrrolidone, or bovine serum albumin added to in vitro suspensions of condensed mitochondria were shown to cause a transformation to the orthodox configuration. A role for outer compartment oncotic pressure was suggested [10]. It was theorized that when mitochondria are removed from the cell, outer compartment oncotic pressure is unmatched by the usual sucrose isolation medium. Therefore, a configurational transformation, orthodox to condensed, takes place to minimize the oncotic pressure differential across the outer membrane. If a matching oncotic pressure is then added to the suspending medium, the transformation is reversed.

The spontaneous orthodox to condensed transformation that occurs during isolation in 0.25 M sucrose can be duplicated in vitro as shown by the micrographs of fig.2. When condensed, freshly isolated rat liver mitochondria (fig.2a) are resuspended in 100 mM sucrose, they transform to a predominantly orthodox form by osmotic expansion of the inner compartment (fig.2b). However, after several minutes' incubation at room temperature there is a spontaneous reversion to the condensed configuration (fig.2c) analogous to the spontaneous orthodox to condensed transformation that occurs during isolation.

Mitochondria undergo energy-linked as well as passive configurational transformations as originally demonstrated [3]. During incubation under respira-

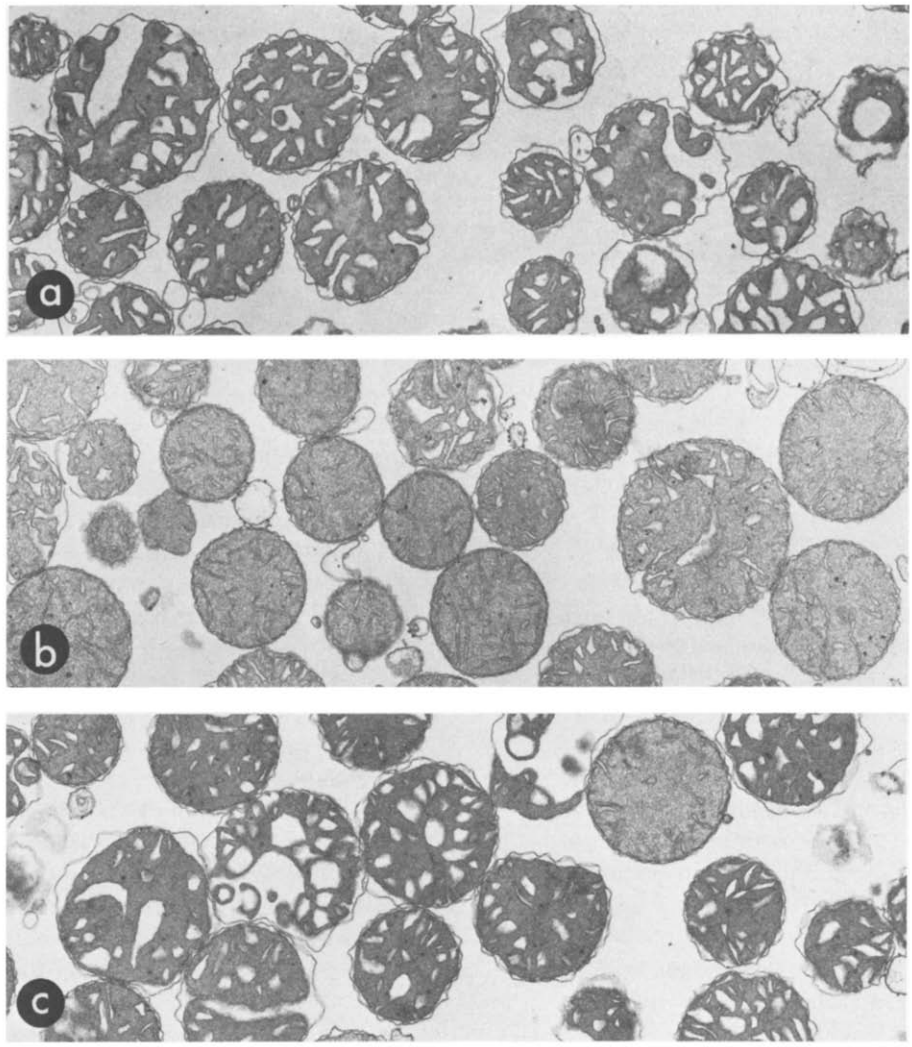


Fig.2. Electron microscopy of rat liver mitochondria. In (a) freshly isolated mitochondria show the condensed configuration. In (b) mitochondria were fixed immediately after resuspension in 100 mM sucrose, 4 μM rotenone. They display a predominantly orthodox configuration although partially condensed and broken mitochondria are also present. In (c) a mitochondrial suspension identical to (b) was incubated for 20 min at 23°C before being fixed. There is a return to a predominantly condensed configuration. X 20 000.

tory state 4 conditions isolated rat liver mitochondria transform from a condensed to an orthodox configuration. In the orthodox configuration the oncotic pressure of the outer compartment is high, and since there is no added oncotic component to the suspending medium, one may conclude from the preceding considerations that there is a net osmotic pressure gradient across the outer membrane. Since the inner and outer compartments have identical osmotic pressures, the entire mitochondrion is hyperosmotic with respect to the suspending medium. If these energized orthodox mitochondria are subsequently de-energized by ADP [3], anaerobiosis, or uncouplers [12,13], there is a rapid return to the condensed configuration. Since the outer compartment has expanded, its oncotic pressure is reduced by roughly 7-fold. Therefore, each mitochondrion is less hyperosmotic to the same degree. In these in vitro experi-

ments, I propose that mitochondria become hyperosmotic to the suspending medium in an energy-linked fashion, presumably by active ion accumulation. The result is a condensed to orthodox configurational transformation. When the energy source is removed, the osmotic gradient dissipates, and an orthodox to condensed transformation ensues by the same outer compartment oncotic pressure mechanism proposed [10] to explain orthodox to condensed transformations occurring during isolation procedures. This is schematically summarized in fig.1.

Energy-linked configurational transformations are observed in situ as well as in vitro [14–16] indicating that such changes are not simply in vitro artifacts. These results suggest that orthodox mitochondria in situ are also hyperosmotic to their intracellular milieu.

If the oncotic pressure hypothesis is correct, then the mitochondrial outer membrane has a special structural role. In a manner somewhat analogous to the bacterial cell wall, the outer membrane supports an osmotic gradient that, if unresisted, would cause osmotic swelling and lysis of the organelle. This postulated mechanical property of the outer membrane can be inferred from the osmotic swelling experiments of fig.3. Although mitochondria are well known to behave as ideal osmometers, some investigators have noted this ideal behavior only after breakage of the outer membrane [7,17]. Figure 3 compares the osmotic behavior of intact mitochondria and mitochondria subjected to hypotonic shock in 45 mM sucrose. The latter treatment breaks the outer membrane but leaves the inner membrane intact and the inner compartment osmotically active. The two types of mitochondria are resuspended in sucrose solutions of different osmolarities and A_{540} is measured immediately afterwards. This measurement is inversely proportional to inner compartment volume if it is corrected for the refractive index of the suspending medium [7]. When the data are plotted as 1/osmolarity versus $1/A_{540}$, a straight line indicative of ideal osmotic behavior is formed for hypotonically shocked mitochondria but not for intact mitochondria. Close examination of the data for intact mitochondria reveals that these mitochondria swell less per increment of osmotic change than do shocked mitochondria in the range from 500–100 mOs. From 100–50 mOs the intact mitochondria swell more rapidly. This correlates with the breakage of the outer membrane, since at 100 mOs most of the mitochondria are in the orthodox configuration (fig.2b), and further osmotic expansion cannot take place without outer membrane breakage. From 50–20 mOs the intact and shocked mitochondria behave identically, apparently because all the outer membranes of the intact mitochondria are broken. These data indicate that mitochondria swell less than expected when the outer membrane is intact and more than expected as the outer membrane bursts which supports the hypothesis that the outer membrane can resist osmotic expansion of the inner compartment.

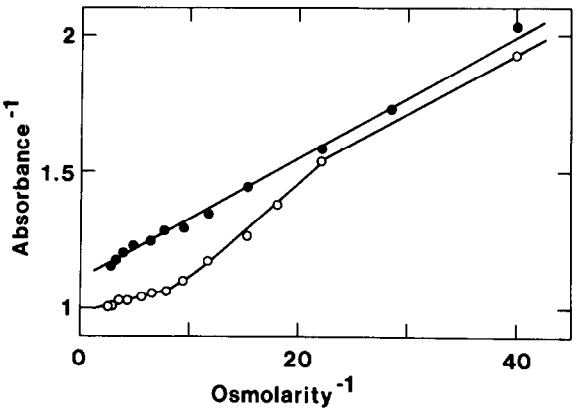


Fig.3. Osmotic swelling properties of mitochondria with intact (o–o–o) and broken (•–•–•) outer membranes. Intact rat liver mitochondria or mitochondria first swollen in 45 mM sucrose were resuspended in various concentrations of sucrose. A_{540} was measured and corrected for the refractive index of the suspending medium [7].

4. Conclusion

Consideration of the permeability characteristics of mitochondrial membranes leads to the conclusion that a significant osmotic pressure gradient can exist across the mitochondrial outer membrane. This pressure gradient is equal to the difference between outer compartment and external oncotic pressures and may be as great as 20 mOs or more when mitochondria assume an orthodox configuration. It is suggested that these osmotic and oncotic pressure

gradients participate in the morphological changes observed in mitochondria during in vitro isolation and during changes in metabolic state. Since an osmotic force tending to expand the inner compartment will also tend to cause an increase in outer compartment oncotic pressure that opposes expansion, it would appear that the oncotic pressure hypothesis provides a mechanism for the regulation and control of mitochondrial volume. The oncotic pressure hypothesis proposes that mitochondria are able to maintain a hyperosmotic internal milieu. As a result, the outer membrane has a special structural role in preventing osmotic swelling and lysis of the organelle.

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