

## ON THE MECHANISM OF MERCURIAL-INDUCED PERMEABILITY OF THE MITOCHONDRIAL MEMBRANE TO $K^+$

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### 1. Introduction

The inner mitochondrial membrane under normal conditions exhibits a low permeability to monovalent cations, in particular to  $K^+$  (reviewed in [1,2]). This can be increased by  $Hg^{2+}$  and organic mercurials. Although this effect has been long known [3–8], its mechanism is far from being understood. A hypothesis was put forward [9,10] that endogenous mitochondrial ionophores for monovalent and divalent cations described [11–13], normally latent in isolated mitochondria, are unmasked by mercurials. On the other hand, permeability changes induced by mercurials in the inner mitochondrial membrane have been shown [7,14] to depend on the amount of thiol groups blocked and the extent of penetration of the mercurial into the membrane. This suggests that a distortion produced by the agent in the membrane proteins rather than mobilization of latent ionophores may be responsible for the increased permeability.

Most of these studies have been performed on heart mitochondria whose permeability characteristics, especially with respect to  $Mg^{2+}$ , somewhat differ from those of liver mitochondria [1,2]. It became interesting therefore to see whether liver mitochondria react in a similar way to mercurials as do heart mitochondria. The results obtained during this study led us to the conclusion that mercurials produce a depletion of tightly bound  $Mg^{2+}$  from the inner mitochondrial membrane and that this results in an increased permeability to monovalent cations and, in particular, to a monovalent cation/ $H^+$  exchange as described in [15].

*Abbreviations:* PHMB, *p*-hydroxymercuribenzoate; CCCP, carbonyl cyanide-*m*-chlorophenylhydrazone

### 2. Methods

Mitochondria were isolated from livers of albino rats by a conventional procedure [16]. Swelling of mitochondria was followed by recording  $A_{520}$  or  $A_{540}$  in a cuvette of 1 cm lightpath containing about 0.5 mg mitochondrial protein in 3.0 ml total vol., at room temperature. Fluorescence of chlorotetracycline was measured at 380 nm excitation and 520 nm emission wavelengths. Mitochondrial  $Mg^{2+}$  was determined by atomic absorption.

### 3. Results and discussion

Under experimental conditions PHMB at the concentration of 12.5 nmol/mg protein produced a 50% release of mitochondrial  $Mg^{2+}$  during 7 min incubation at 20°C but had little effect on mitochondrial swelling in either isotonic KCl or  $KNO_3$  unless an uncoupler, like 2,4-dinitrophenol or CCCP, was added (fig.1A,B). This can be interpreted as indicating that the mercurial promotes an electroneutral  $K^+/H^+$  exchange across the inner mitochondrial membrane [15]. An alternative explanation that the swelling results from blocking, by the uncoupler, the energy-dependent extrusion of  $K^+$  [17] is not tenable under these experimental conditions, since respiratory inhibitors rotenone, antimycin and cyanide did not produce swelling.

It is also evident that PHMB induced the permeability to  $Cl^-$  as indicated by swelling in KCl solution. These effects of mercurials on the monovalent cation/ $H^+$  exchange and permeability to  $Cl^-$  have already been observed in heart mitochondria [6,8,10].

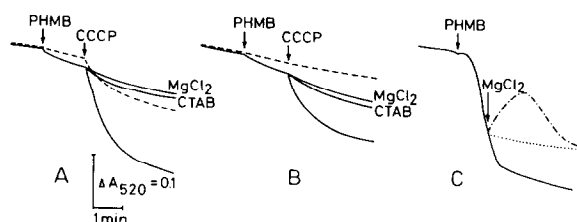


Fig.1 Swelling of mitochondria induced by PHMB. The incubation media were buffered with 10 mM Tris-HCl (pH 7.4) and contained: A, 130 mM  $\text{KNO}_3$ ; B,C, 125 mM KCl. In A and B the media also contained 3.2 mM  $\text{MgCl}_2$  or cetyltrimethylammonium bromide (CTAB) 30 nmol/mg protein, where indicated. PHMB 12.5 nmol/mg protein and 1  $\mu\text{M}$  CCCP were added at arrows. Dashed lines represent samples to which no PHMB was added. In C, PHMB was 40 nmol/mg protein, and the final concentration of  $\text{MgCl}_2$  added at the arrow was 3.2 mM (· · ·) or 20 mM (---).

The PHMB-induced swelling could be prevented by 3 mM  $\text{MgCl}_2$  (fig.1) or  $\text{MnCl}_2$  (not shown). The cationic detergent, cetyltrimethylammonium bromide, also prevented the PHMB-induced swelling, even at much lower concentrations than  $\text{MgCl}_2$  and  $\text{MnCl}_2$  (fig.1).

At 40 nmol/mg protein, PHMB induced a rapid swelling of liver mitochondria in both KCl and  $\text{KNO}_3$ , even in the absence of uncoupling agents. This swelling was also prevented by  $\text{Mg}^{2+}$ . If  $\text{MgCl}_2$  was added during the swelling phase, the process was arrested or partly reversed, depending on the concentration of  $\text{Mg}^{2+}$  added (fig.1C).

To see whether PHMB also increased the permeability of the inner mitochondrial membrane to  $\text{Mg}^{2+}$ , swelling of mitochondria in isotonic  $\text{Mg}(\text{NO}_3)_2$  was measured. As can be seen in fig.2, the swelling starts after a lag of about 7 min following addition of PHMB. In the presence of an uncoupler the onset of swelling was immediate and the rate increased with time. The following conclusions can be drawn from these observations:

- (i) The mercurial mobilizes a  $\text{Mg}^{2+}/\text{H}^+$  exchange;
- (ii) Activation of the  $\text{Mg}^{2+}/\text{H}^+$  exchange proceeds much slower than in case of the  $\text{K}^+/\text{H}^+$  exchange (fig.1) and requires, most likely, a deeper penetration of the mercurial into membrane proteins;
- (iii) A prolonged incubation of mitochondria with the mercurial makes the membrane permeable to

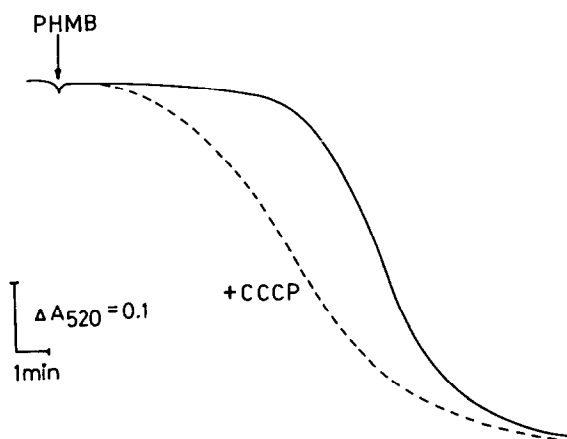


Fig.2. Swelling of mitochondria in magnesium nitrate. The medium contained 100 mM  $\text{Mg}(\text{NO}_3)_2$  and 10 mM Tris-HCl (pH 7.4); 1  $\mu\text{M}$  CCCP was also present in the sample represented by the dashed line. PHMB was 50 nmol/mg protein.

$\text{H}^+$ , as manifested by the loss of an absolute requirement for the uncoupling agent.

The latter conclusion could also be drawn from the effect of higher PHMB concentration on the swelling in KCl (fig.1C).

It has been observed [5,7,14] that removal of bound mercurials by compounds containing reactive thiol groups, e.g., cysteine or dithioerythritol, can reverse some of the permeability changes produced in mitochondria. To see whether a simple removal of the mercurial is sufficient or other factors are also needed, the following experiment was performed. Mitochondria pretreated with PHMB were washed with sucrose solution containing 2-mercaptoethanol. Such mitochondria were no longer permeable to  $\text{Mg}^{2+}$ , as shown by the lack of swelling in isotonic  $\text{Mg}(\text{NO}_3)_2$  in the presence of an uncoupler (fig.3, c.f. fig.2). However, they still exhibited a rapid swelling when added to 125 mM KCl, the swelling being inhibited by 5 mM  $\text{MgCl}_2$ . This experiment shows that, upon removal of the mercurial, the increased  $\text{Mg}^{2+}/\text{H}^+$  exchange is blocked whereas the  $\text{K}^+/\text{H}^+$  exchange and probably the increased permeability to  $\text{H}^+$  persist.

The effect of PHMB on the binding of  $\text{Mg}^{2+}$  to mitochondrial membranes was studied by measuring the fluorescence of chlorotetracycline [18,19]. Figure 4 shows that even in the presence of 5 mM

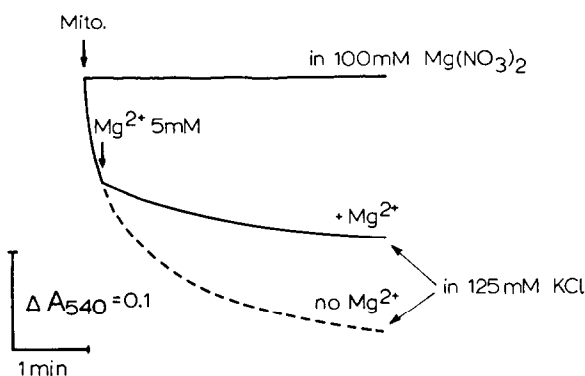


Fig.3. Swelling of mitochondria after preincubation with PHMB and washing with mercaptoethanol. Mitochondria were pretreated with PHMB 50 nmol/mg protein during 3 min at 0°C and subsequently washed with cold 250 mM sucrose containing 0.1 mM 2-mercaptoethanol. Composition of the swelling media and additions were as indicated. The media were buffered with 10 mM Tris-HCl (pH 7.4); the  $\text{Mg}(\text{NO}_3)_2$  medium also contained 1  $\mu\text{M}$  CCCP.

$\text{MgCl}_2$  in the medium, phosphate and CCCP produce a decrease of the fluorescence, indicating a release of membrane-bound  $\text{Mg}^{2+}$ . The effect of phosphate is identical with that observed in heart mitochondria [20] and interpreted as indicative for a shift of  $\text{Mg}^{2+}$

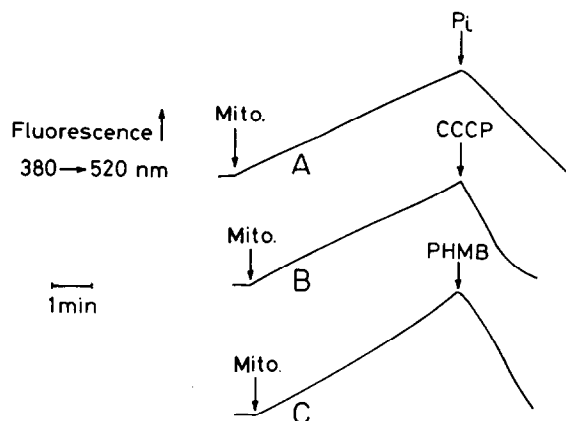


Fig.4. Effect of PHMB, phosphate and CCCP on chlorotetracycline fluorescence in mitochondria. Mitochondria (0.1 mg protein/ml) were incubated at 20°C in the medium containing 250 mM sucrose, 20 mM Tris-HCl (pH 7.4), 2.5 mM succinate, 2  $\mu\text{M}$  rotenone, 5 mM  $\text{MgCl}_2$  and 10  $\mu\text{M}$  chlorotetracycline. The additions were as follows (final concentrations): 5 mM phosphate, 1  $\mu\text{M}$  CCCP, PHMB 50 nmol/mg protein.

from the inner membrane to the matrix compartment, whereas the effect of CCCP is probably due to a release of  $\text{Mg}^{2+}$  into the external medium. Trace C of fig.4 shows that PHMB also induces a rapid decrease of chlorotetracycline fluorescence which, likewise, indicates a depletion of the mitochondrial membrane of membrane-bound  $\text{Mg}^{2+}$ . However, attempts to assess this depletion by a direct analysis were unsuccessful because of a low content of  $\text{Mg}^{2+}$  in isolated mitochondrial membranes [21] and also because the separation procedure alone probably produces a partial loss of loosely bound  $\text{Mg}^{2+}$ .

Thus, the present results strongly suggest that the increase of permeability of the inner mitochondrial membrane to monovalent cations, as induced by PHMB and other mercurials, is a result of the depletion of membrane-bound  $\text{Mg}^{2+}$ . A release of mitochondrial  $\text{Mg}^{2+}$  by thiol-oxidizing agents has already been observed [22] but the mechanism by which thiol groups promote binding of  $\text{Mg}^{2+}$  in or on the mitochondrial membrane remains obscure. It is likewise not clear whether the primary effect of the depletion of membrane-bound  $\text{Mg}^{2+}$  is a promotion of the electroneutral monovalent cation/ $\text{H}^+$  exchange as suggested [15] or of the electrophoretic permeability to monovalent cations as postulated [23]. A third possibility has recently been proposed [24], namely that the release of  $\text{Mg}^{2+}$  leads to a primary increase of  $\text{H}^+$  permeability which becomes then short-circuited with  $\text{K}^+$  antiport.

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