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The permeability transition pore. Control points of a cyclosporin A-sensitive mitochondrial channel involved in cell death

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Abstract

The permeability transition pore (MTP) is a high conductance channel of the mitochondrial inner membrane inhibited by cyclosporin A. While the physiological role of the MTP has not been clarified yet, it is becoming clear that this channel plays an important role in the pathways leading to cell death. The recent demonstrations that the MTP is controlled by the membrane potential, that a variety of physiological and pathological effectors can modulate the threshold voltage at which pore opening occurs, and that surface potential may contribute to pore modulation provide a useful framework to describe the mechanistic aspects of pore function in isolated mitochondria. Here we (i) briefly review the key features of pore regulation, and report our recent progress on the role of oxidants and mitochondrial cyclophilin; and (ii) elaborate on how MTP regulation by cellular pathophysiological effectors (such as cytosolic $[Ca^{2+}]$ transients, oxidative stress, and changes in the concentration of polyamines, nitric oxide, and metabolites of both the sphingomyelin and phospholipase A_2 pathways) might take place in vivo. Further definition of the MTP checkpoints should help in the design of specific modulators, and offers great promise for the development of new conceptual and pharmacological tools aimed at therapeutic intervention in pathological conditions where pore opening is a critical event.

Keywords: Mitochondrion channel; Cyclosporin cell death

1. Introduction

After accumulation of Ca^{2+}, isolated mitochondria easily undergo a permeability increase (the 'permeability transition', PT) to solutes with molecular masses up to 1500 Da through pores with an estimated diameter of about 3 nm [1]. This leads to collapse of the proton electrochemical gradient, and therefore to cessation of ATP synthesis [2]. The ensuing Ca^{2+} release, osmotic swelling, and hydrolysis of any available ATP by the F_1F_0 ATPase have historically linked this phenomenon to mitochondrial dysfunction [3]. This partly explains why substantial progress has been made towards an understanding of the role of the PT in cellular pathology (e.g., Refs. [4] and [5], and [6] for a review), while its physiological role remains elusive (but see Refs. [7] and [8]).

Under prevailing in vitro conditions the pores display a lack of *selectivity* for the permeating species, and the only apparent discrimination between solutes is their molecular size. In the past, this property has been implied to mean that the pathway for permeabilization lacked *specificity*, i.e. it was not mediated by a protein designed for a physiological function [3]. The electrophysiological identification of mitochondrial channels [9], the discovery that CSA is a high-affinity inhibitor of the PT, and the recent definition of the key control points of this complex phenomenon have drastically changed this picture (see Ref. [10] for a review). The field has come to agree that the PT is mediated by opening of a *specific* pathway (the MTP, a regulated inner membrane channel), which coincides with the mitochondrial megachannel independently identified by electrophysiological studies [9]. The fully opened state of the channel in the high-conductance (1 nS) mode appears to correspond to the unselective state of the MTP observed in isolated mitochondria in vitro and in intact

Abbreviations: PT, permeability transition; MTP, mitochondrial permeability transition pore; CSA, CSH, cyclosporin A and H, respectively; Cyp-M, mitochondrial cyclophilin.

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cells under pathological conditions, while the frequent subconductance states displayed in single-channel measurements [9] might correspond to a more solute-selective state stabilized by CSA which has been recently described in isolated mitochondria [11]. In vitro MTP opening is fully reversible [12]. After pore closure mitochondria repolarize recovering their energy-linked functions, and under proper conditions they regain their initial volume by solute extrusion [13]. Pore reversibility and its tight control by both the membrane potential and by matrix pH link its operation to the basic events of energy conservation, and suggest a physiological function which may be related to Ca^{2+} homeostasis [8]. These advances confirm the pioneering studies of Hunter and Haworth, who were the first to identify the 'transitional' nature of the permeability increase, and to argue that opening of a proteinaceous pore was responsible for this phenomenon [14–16].

Here we report our current understanding of the mechanistic aspects of pore regulation, and elaborate on how it could be modulated by intracellular signals. Due to severe space constraints, with few exceptions we refer the reader to recent reviews for papers published before 1994 [3,6–10].

2. Mechanistic aspects of pore regulation

2.1. Membrane potential

A useful description of many known pore responses to physiological and pathological signals can be made within the framework of its voltage-dependence, which was recognized in 1992 [17]. The channel favors the closed state as the voltage becomes more negative inside, i.e. under physiological conditions. This appears to be an intrinsic property of the MTP which can be observed both in isolated mitochondria (by modulating the membrane potential with uncouplers) and in patch-clamp experiments of single channels (by modulating the applied voltage) (see Refs. [10] and [9], respectively, for reviews). These findings led us to postulate the existence of a sensor which decodes the voltage changes into variations of the probability of pore opening [10]. Irrespective of the molecular structure of the putative sensor, it appears that many effectors are able to modify the threshold voltage (the 'gating potential') at which MTP opening occurs. Many pore agonists (for example oxidative agents) shift the apparent gating potential to more negative (physiological) values, thereby favoring pore opening, while many pore antagonists (like reducing agents) have the opposite effect and favor its closure [10]. Thus, pore opening can be obtained by either depolarization, or by changing the threshold potential at which opening occurs.

2.2. Surface potential

The recent proposal that the pore also senses changes of the surface potential may represent a substantial improvement in our understanding of how MTP responses are modulated by a variety of heterogeneous agents [10,11]. Amphipathic anions (like fatty acids) behave as pore activators with an effect that cannot be explained by depolarization [11]; and conversely, polycations (like spermine, Ref. [18]), positively charged peptides [19] and amphipathic cations (like sphingosine and trifluoroperazine) inhibit pore opening, the latter acting independently of inhibition of phospholipase A_2 [11]. The available data are consistent with the view that a more positive surface potential favors pore closure, while a more negative surface potential favors its opening. This hypothesis also resolves the puzzling observation that atractylate and bongkrekate, both inhibitors of the adenine nucleotide translocase, affect the pore in opposite directions. Atractylate, which stabilizes the translocase in the 'c' conformation, favors pore opening while bongkrekate, which stabilizes the translocase in the 'm' conformation, favors pore closure. Since the 'm' to 'c' transition profoundly affects the surface potential in the expected direction, the effects of atractylate and bongkrekate on the MTP find an elegant explanation within the framework of pore modulation by the potential [11].

2.3. Matrix pH, CSA and MTP modulation by Cyp-M membrane interactions

The MTP is potently inhibited by H^+ [14]. The effect of H^+ is exerted from the matrix side of the inner membrane, and is linked to reversible protonation of histidyl residues [20]. The pore is extremely sensitive to variations of matrix pH, in that at pH below about 7.0 the pore is substantially inhibited while at pH 6.5 it does not open even in fully depolarized mitochondria [18]. The pore is also inhibited by nanomolar concentrations of CSA, and this effect is most likely mediated by Cyp-M, a member of the cyclophilin family of peptidyl-prolyl-*cis-trans*-isomerases selectively localized to the mitochondrial matrix [21]. Based on recent findings, we propose that the inhibitory effect of both H^+ and CSA is due to unbinding of Cyp-M from the pore, which would result in increased probability of pore closure [22]. This hypothesis is supported by the findings that (i) Cyp-M can be released from submitochondrial particles by CSA or by H^+ but not by CSH, and the effect of acidic pH is prevented by diethylpyrocarbonate; this matches MTP inhibition, which can be observed with CSA but not CSH, and with the relief of H^+ inhibition by diethylpyrocarbonate [20]; and (ii) that reaction of mitochondria with phenylarsine oxide, a powerful pore agonist, increases the affinity of binding of Cyp-M to mitochondrial membranes [23]. Thus, many agonists and antagonists

might modulate the MTP by affecting its interactions with Cyp-M.

2.4. Pore modulation by redox agents at two separate sites

Oxidative stress has long been known to increase the probability of pore opening, but the precise mechanism remains obscure [9]. In general, MTP opening is favored by oxidants of both pyridine nucleotides (like acetoacetate and oxaloacetate), glutathione (like *tert*-butylhydroperoxide), and of dithiols (like diamide), as well as by dithiol crosslinkers (like phenylarsine oxide and arsenite). Since pyridine nucleotides and glutathione levels are connected through transhydrogenase and glutathione reductase, it was not clear whether these oxidative events could be linked by a common mechanism [9]. After pointing out the importance of dithiol-disulfide interconversion in the modulation of the MTP response to depolarization [24,25], quite recently we have been able to readdress this long-standing issue by independently modulating the levels of reduced pyridine nucleotides and glutathione in both energized [26] and deenergized [27] mitochondria. The results of our studies indicate that two sites can be experimentally distinguished, both contributing to MTP modulation by oxidating and reducing agents.

(i) A first site (which we call the 'S-site') coincides with the oxidation-reduction sensitive dithiol [25]. Cross-linking of the S-site by arsenite or phenylarsine oxide, or its oxidation by oxidized glutathione is matched by increased MTP open probability under conditions where the pyridine nucleotides pool is kept in the fully reduced state [26,27]. This site can be blocked by both *N*-ethylmaleimide and monobromobimane, and the effects of cross-linking or oxidation can be fully reverted by reductants like dithiothreitol. As indicated in the schematic picture reported in Fig. 1, glutathione appears to be the immediate oxidant of the S-site, and many pore agonists (like organic hydroperoxides) affect it through changes in the levels of reduced glutathione rather than by direct oxidation [27].

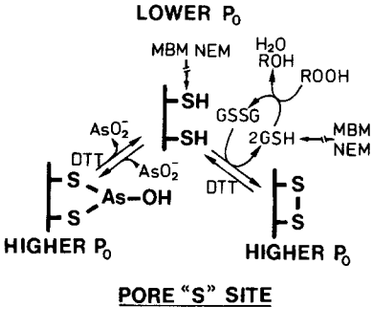

Fig. 1. Modulation of the MTP open probability at the S-site. The scheme illustrates how the MTP open probability is affected by the S-site, a critical dithiol. A lower open probability is associated with the reduced form, and can be stabilized by reaction with both *N*-ethylmaleimide (NEM) or monobromobimane (MBM). The dithiol can be reversibly oxidized by oxidized glutathione, and a higher open probability is associated with the disulfide. Finally, the dithiol can react with arsenite (or phenylarsine oxide, not shown). The cross-linked species is equivalent to the disulfide, and is matched by an increased open probability which can be reversed by dithiothreitol (DTT). The scheme is based on the experiments discussed in the text and described in detail in Refs. [26] and [27].

(ii) A second site (which we call the 'P-site') is in apparent oxidation-reduction equilibrium with the pyridine nucleotides pool. Pyridine nucleotides oxidation is matched by increased MTP open probability under conditions where the glutathione pool is kept in the fully reduced state, or when the dithiol is reacted with arsenite. At variance from the S-site, this site cannot be blocked by monobromobimane or dithiothreitol, while it is sensitive to *N*-ethylmaleimide [26]. Since the chemical reactivity of the S-site towards monobromobimane is unaffected when pyridine nucleotides are oxidized by a variety of treatments, we conclude that the two sites do not share common oxidation-reduction intermediates besides glutathione itself [27].

2.5. Two general mechanisms for MTP modulation?

From the identification of the above control points, a general picture emerges which may account for most known pore agonists and antagonists within the relatively simple framework of the MTP as a voltage-dependent channel activated by matrix Ca^{2+} and modulated by Cyp-M membrane interactions. (i) Modulation by both the membrane and surface potential would be made possible by the voltage sensor, which is presumed to be located at the interface between the membrane and the outer aqueous phase [10]. This would render the pore responsive to changes of the proton motive force (for example, by inhibition of respiration or by uncoupling), to signalling molecules/events occurring in the cell (for example sphingolipids and Ca^{2+} transients) or resulting from metabolism of phospholipids on the mitochondrial membrane itself (such as fatty acids produced by phospholipase A_2 activation), and to oxidative events through the P- and S-sites. (ii) Modulation by Cyp-M binding would be afforded by conformational changes effected by protonation or CSA, and would convey signals arising from the matrix. These signals may involve inner-outer membrane interactions at the contact sites [28], and possibly hitherto unsuspected biochemical signalling pathways.

The idea that two classes of pore agonists can be distinguished – one acting through the sensor and one through Cyp-M – is supported by the finding that, depending on the agonist, in long time-frame experiments the MTP can be either fully sensitive to trifluoroperazine but not CSA, or fully sensitive to CSA but not trifluoroperazine, and that with several agonists a long-lasting inhibition can only be obtained when trifluoroperazine and CSA are added together, with a clear synergistic effect [11].

3. Pore modulation by cellular signals

It is now clear that mitochondria play a major role in the regulation of intracellular Ca^{2+} homeostasis [29–31],

and that they possess a mechanism which is kinetically competent for the efficient sequestration of Ca^{2+} pulses in the submicromolar range [32]. It appears that Ca^{2+} signalling to the pore is a feasible mechanism by which pathological conditions characterized by Ca^{2+} overload may cause mitochondrial dysfunction, such as the ischemia-reperfusion syndrome (see, e.g., Refs. [33–35]). Potentiation of the Ca^{2+} signal could be achieved by activation of phospholipase A_2 through the production of free fatty acids, which may affect the pore open-closed transitions through changes of the mitochondrial surface potential [11].

Monovalent reduction of oxygen by the mitochondrial respiratory chain produces superoxide anions, which then yield hydrogen peroxide through the superoxide dismutase reaction. The latter species oxidizes mitochondrial glutathione, and is therefore a likely candidate as an endogenous mediator which can affect MTP open-closed transitions at the S-site [36]. A similar mechanism can explain the cellular toxicity of organic hydroperoxides like *tert*-butylhydroperoxide [37] and of compounds undergoing redox cycling like menadione [24] and paraquat [38]. It is intriguing that depending on the dose a redox-cycling quinone can stimulate cell growth, trigger apoptosis, or cause cell necrosis through a mechanism which involves changes in the synthesis of polyamines and of cytosolic $[Ca^{2+}]$[39], and that cell killing by tumor necrosis factor involves overproduction of oxygen radicals by the mitochondrial respiratory chain [40], and is mediated by signalling through the sphingomyelin pathway which also yields mediators acting on the pore [10,11]. Finally, important synergistic effects have been observed between paraquat and nitric oxide [38], which may indirectly affect pore function through its reversible inhibition of cytochrome oxidase [41].

4. The pore and cell death

Overwhelming evidence indicates that opening of the MTP is a key event in a variety of toxic, hypoxic and oxidative forms of cell injury [4–6,33–35,37], suggesting that pore dysregulation can be a final common pathway on which many noxius agents or conditions converge. The intriguing observation that the pore might also be involved in apoptosis [7] and the potential role of oxygen radicals as mediators of the death signal(s) in a variety of diseases of both genetic (like muscular dystrophy, Ref. [42]) and infectious nature (like AIDS, Ref. [43]) is rapidly extending the interest on mitochondrial dysfunction in general, and on this channel in particular, to immunology, oncology and pathology. The definition of the mechanistic aspects of pore modulation [10], the demonstration that both glutathione and pyridine nucleotides oxidation affect the probability of pore opening [26,27], and the identification of Cyp-M as a potential target for selective pore inhibition with non-immunosuppressive CSA derivatives [22] should lead to an improvement of our ability to control pore function in vivo, and offer great potential for pharmacological treatment of diseases in which pore opening plays a role.

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