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Inorganic polyphosphate (polyP) as an activator and structural component of the mitochondrial permeability transition pore

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Abstract

Mitochondrial permeability transition pore (mPTP) is a large channel located in the mitochondrial inner membrane. The opening of mPTP during pathological calcium overload leads to the membrane depolarization and disruption of ATP production. mPTP activation has been implicated as a central event during the process of stress-induced cell death. mPTP is a supramolecular complex composed of many proteins. Recent studies suggest that mitochondrial ATPase plays the central role in the formation of mPTP. However, the structure of the central conducting pore part of mPTP (mPTPore) remains elusive. Here we review current models proposed for the mPTPore and involvement of polyP in its formation and regulation. We discuss the underestimated role of polyP as an effector and a putative structural component of the mPTPore. We propose the hypothesis that inclusion of polyP can explain such properties of mPTP activity as calcium activation, selectivity and voltage-dependence.

Keywords

calcium; inorganic polyphosphate (polyP); mitochondria; permeability transition

Introduction

In the past few years, considerable attention has been directed towards an investigation of the multiple roles played by polyP in mammalian organisms [1–6]. Due to its molecular nature and its ubiquitous presence, polyP can be potentially involved in a plethora of biological processes. The role of polyP is particularly prominent in mitochondria where, due to the presence of phosphoanhydride bonds, similar to ones found in ATP, it participates in energy homeostasis and is also capable to form complexes with divalent ions, including magnesium and calcium [7]. Of particular interest is the involvement of polyP in the mitochondrial response to pathological conditions. Whereas multiple possible mechanisms

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of polyP participation might exist, molecular details of these processes remain largely unknown. Here we will discuss the molecular mechanisms underlying the contribution of polyP towards the induction of the pathological mitochondrial membrane permeability (permeability transition) that occurs during cellular stress. We will review current experimental data that support the idea of the direct involvement of polyP in the activation and formation of the mitochondrial permeability transition pore (mPTP).

Permeability transition pore phenomenon

Mitochondria play a central role in cellular energy metabolism, as well as in Ca^{2+} and reactive oxygen species (ROS) signalling [8–11]. It has been established that loss of mitochondrial function is a major contributor to stress-induced necrotic and apoptotic cell death [12–15]. In terms of relevant diseases, one of the most significant and well-documented examples is the role of mitochondrial loss of function in tissue damage during the stroke [15–18]. Specifically, it has been found that ischemia occurring during stroke followed by re-oxygenation induces profound mitochondrial damage that causes cell death [19–21]. These observations lead to the paradigm in which the protection and restoration of mitochondrial function during acute stress is pivotal to cell survival. Indeed, this concept has been supported by a vast body of experimental data, ranging from cell culture models to human clinical trials [19,22–26]. Elucidation of the mechanisms responsible for the mitochondrial damage is considered a promising approach for the discovery of novel therapeutics for currently untreatable conditions resulting from apoptotic and necrotic cell death. At the molecular level, a central event responsible for the loss of mitochondrial function is the sudden and dramatic increase in the permeability of the mitochondrial inner membrane. This phenomenon, termed permeability transition, causes dissipation of the electrochemical potential across the mitochondrial inner membrane which is the essential driving force for ATP production by mitochondria and, thus, its loss immediately stops mitochondrial ATP production and, if not corrected in a timely fashion, results in cell death [27–30]. Key factors contributing to the activation of mPTP during acute stress are elevated levels of matrix Ca^{2+} and ROS. Prevention of mPTP opening during stress helps to maintain inner membrane integrity and ATP production. In this important way, inhibition of mPTP opening can prevent cell death [27,29,31–39]. Whereas mPTP in most cases is considered as a pathological event, its possible significance in normal physiology in the heart has recently been proposed[40].

Permeability transition pore structure: current views

Structurally, mPTP is believed to be a supra-molecular complex composed of dozens of proteins [41,42]. Genetic and pharmacological manipulations of various proteins, peripheral members of the complex, have established the proof of principle that mPTP inhibition is protective against cell death. One of the best-described proteins involved in mPTP activation is cyclophilin D. This protein is implicated as a key regulator of mPTP activity and cell death [31,43,44]. More recent studies have identified several proteins that might play a critical role in mPTP activation [45–47]. These putative components of the mPTP include SPG7, p53 and proteins from the Bcl-2 family, as Bax and Bak. SPG7 has been proposed to be a core conserved protein involved in mPTP activation and located at the contact sites

between mitochondrial inner and outer membranes [45]. p53 protein involvement in triggering the mPTP opening is associated with its ability to interact directly with cyclophilin D [46]. Finally, Bax and Bak, channel-forming proteins of the outer membrane are proposed to be required for activation of mPTP opening in the inner membrane in a large pore conformation [47].

Interestingly, the most critical question on the molecular identity of the central 'channel' part of mPTP remains unresolved. Here we will refer to the channel part of the complete mPTP complex as 'mPTPore' (pore part of mPTP). Since the discovery of the mPTP, several protein candidates have been considered to play the part of the mPTPore including adenine nucleotide translocator (ANT)[48], phosphate carrier (PiC) [49] and 'misfolded' proteins[50]. Although experimental data confirm the involvement of these components in mPTP, none of them appear to be essential [51–53], suggesting that they are not the integral part of the mPTPore domain. Recent studies, performed independently in three laboratories, put forward a new concept: the mPTPore is associated with mitochondrial ATPase, a supramolecular complex containing matrix (F1) and membrane (F0) parts and composed of approximately 30 individual proteins [54–56]. However, experimental data currently available do not provide a conclusive mechanism for the mPTPore formation or its molecular composition. Both of these questions are the subject of hot debate (Figure 1 for currently proposed models). Bernardi and colleagues [41,55] propose that mPTPore probably forms in between the two c-ring subdomains of the ATPase. Opposite, recent reports from Jonas and colleagues [42,54] put forward an alternative view. They showed that the mPTPore part of mPTP is probably associated with the C-subunit protein ring of the ATPase. Whereas the evidence for the involvement of C-subunit is supported by a number of experimental observations [54,56], it is also evident that the C-subunit on its own is not sufficient to explain the behaviour of the mPTP channel [41]. Indeed, the peptide sequence C-subunit consists almost entirely of hydrophobic amino acids. Highly hydrophobic peptides, such as C-subunit, even when assembled into oligomers, are not expected to form a structure that will allow passage of water and ions, essential requirement for a high-conductance low-selectivity channel-like mPTPore. Structural studies confirm that C-subunit oligomers are expected to be associated with hydrophobic molecules of lipid nature, rather than with water [57,58]. Another source of controversy regarding the mPTP activation is the nature of F0–F1 interactions during this process. Alavian et al. [54] present an evidence that that these interactions can weaken during mPTP opening. However, Bernardi et al. [41] argue that dissociation of F0 from F1 would require rather harsh conditions, which is very unlikely *in vivo*. It should also be noted that Halestrap [59] suggests that ATPase, ANT and PiC could all be equally important and theorizes that mPTPore exists within the complex at the interface of these proteins, without giving details; however, concerning the mechanism of pore assembly. Finally, very recently an alternative hypothesis that does not require the involvement of C-subunit has been put forward [45]. Overall, despite significant recent progress the subject of the molecular nature of the mPTPore currently is not well resolved.

Permeability transition pore activation by polyP

Most of the recent efforts in the investigation of the mPTP have been focused on identification of its protein components. Interestingly, even with recent additions of the

protein components of ATPase to the picture, key the properties of the mPTPpore remain unexplained. For example, it is well established that mPTP activation is sensitive to the concentration of free matrix calcium, the condition that is expected to occur during stress related to ischaemic injury [60–62]. However, at the level of the intact mitochondria, mPTP activation can be achieved by the accumulation of calcium inside the matrix without the increase in the concentration of free calcium [63]. These experiments suggest that, contrary to general belief, mPTP is not directly activated by calcium. Electrophysiological recordings demonstrate that mPTP is a channel with well-defined conductance states, selectivity pattern and voltage-dependence [64,65]. This indicates the presence of the core structure that can form stable conducting pathway and presumably contains a number of functional groups responsible for characteristic channel properties. Although the ‘ring’ formed by C-subunit oligomers cannot form a complete channel on its own, considering its structure it can provide the backbone for a conducting pore.

It is possible that the above mentioned apparent controversies can be resolved if non-protein components of mPTP complex are taken into consideration. In the past, some of the non-protein components have been implicated in mPTP activity, including non-esterified fatty acids [66–68] as well as mPTP activation through interactions that involve phospholipid bilayers [69]. However, lipid-based pores are not expected to form stable and well-defined channels as in the case of mPTP (reviewed recently in [70]). On the other hand, polyP is known to be an integral and essential part of fully synthetic ion channels in lipid bilayers [71]. In living organisms, polyP is known to be a central participant in calcium signalling [4], as well as being a structural component of biological ion channels [72–74]. In mammalian mitochondria, polyP is found to be associated with highly purified channel fraction that resembles native mPTP when reconstituted into lipid bilayers (BLM). Involvement of polyP in mPTP has also been confirmed in experiments with living cells. When mitochondria are depleted of polyP, they either do not undergo calcium-induced mPTP or its opening is significantly delayed. In these experiments in cultured cells, polyP was hydrolysed by targeted mitochondrial overexpression of the specific polyphosphatase. Under these conditions, despite active calcium uptake, mitochondria did not develop mPTP. Importantly, similar results were found for different cell types, including stable cultured cells as well as neuronal and cardiac primary cultures [75–78].

Taking into account the multiple roles played by polyP in cell physiology, it is most likely that PolyP regulation of mPTP occurs via several independent mechanisms. PolyP is involved in mitochondrial energy metabolism and activity of the respiratory chain [75,79]. Thus, polyP can modulate the mPTP activation through indirect effects on mitochondrial bioenergetics. More direct effects of polyP might involve its potential ability to contribute towards calcium–phosphate interactions [63]. This property of polyP has not been investigated in the context of mitochondria but has been demonstrated in tissue calcification [80]. mPTP activation requires accumulation of significant amounts of calcium inside mitochondrial matrix. This accumulation does not change the free calcium concentration but leads to the increase in the amounts of calcium-phosphate precipitates of unknown forms [81]. Thus, it is possible that the lack of polymerized form of phosphate might shift the nature and amounts of the calcium precipitates and, by doing so, change the amount of calcium required for mPTP activation.

Can polyP be a structural part of mPTPpore?

In addition to the above-mentioned indirect roles of polyP in mPTP activation, polyP can be directly involved as an essential structural component of mPTPore. It has been demonstrated before in experiments with synthetic polymers, as well as with polymers of bacterial origin that polyP in combination with polyhydroxybutyrate (PHB) can form stable ion channels [71,73]. In mammalian mitochondria, PHB has been implicated as an endogenous ionophore that can be involved in calcium transport [82–84]. Interestingly, in bacteria dramatic increase in this channel assembly is stimulated by the addition of calcium [73,85]. In mammalian organisms, the essential participation of polyP was demonstrated for protein-based TRPM8 channel [72]. Channels formed by the complex of polyP and PHB have also been purified from mammalian mitochondria that were pre-treated with calcium. Importantly, this highly purified mitochondrial extract was essentially protein-free, except of C-subunit of the ATP-synthase. The channel activity of this highly purified complex was not sensitive to the cyclosporine A due to the absence of cyclophilin D but resembled all the key electrophysiological features of the mPTPore channel as seen in native mitochondrial membranes [86]. It is conceivable that in the case of mammalian mitochondria, the formation of mPTP might occur through a calcium-induced assembly of the channel forming complex made of C-subunit, PHB and polyP. Putative model illustrating of how these assemblies might occur during calcium overload is presented in Figure 2. It should be noted that dissociation of F1 from F0 proposed by Alavian et al. [54] and adapted for Figure 2 might not necessarily take place [41]. However, during stress conditions, morphology of the F0–F1 complex could undergo significant changes. Involvement of polyP can explain the phenomenon of calcium activation of mPTP, through the involvement of calcium–phosphate interactions. Further, the presence of polyP and PHB provides an explanation on how a highly hydrophobic protein such as C-subunit can participate in the formation of ion water filled ion-conducting pore. In this scenario, the amphipathic PHB polymer can modify C-subunit in the inside part of the ‘ring’ and, by folding, provide a hydrophilic centre that can interact with calcium and polyP providing a microenvironment that allows ion passage by the pore mechanism (Figure 2). Finally, as a highly charged polymer, polyP is capable of playing a role as the voltage sensor and selectivity filter of mPTP. It is noteworthy that removal of polyP from TRPM8 channel dramatically changes voltage-sensitivity of this channel [72].

Conclusions

In conclusion, we propose the hypothesis that essential role of polyP in mPTP activation might be explained by its direct participation in the formation of mPTPore. Participation of polyP will involve calcium-induced interactions with protein parts of ATPase, most probably its C-subunit. As a highly charged structural component of mPTP, polyP could hypothetically function as the voltage sensor of mPTPore and be responsible for its selectivity. Further studies are needed to confirm the molecular details of polyP involvement in mPTP ion channel function.

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Abbreviations:

ANT	adenine nucleotide translocator
mPTP	mitochondrial permeability transition pore
mPTPore	pore part of mPTP
PHB	polyhydroxybutyrate
PiC	phosphate carrier
ROS	reactive oxygen species

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Figure 1 |. Proposed mechanisms of the mPTP transition from closed to the open state (A) mPTPore is formed at the intersection between ANT, PiC and ATPase [49]. (B) mPTPore is formed within the C-ring of ATPase [45]. (C) mPTP is formed between two monomers of the ATPase [46].

Figure 2 |. Putative mechanism of the polyP participation in mPTPore

(A) Under normal conditions (mPTP closed) ATPase complex is fully assembled with PHB associated with the C-subunit and F1 complex. (B). During pathological conditions, Ca²⁺ and polyP induce dissociation of F1 from C-ring and formation of the mPTPore complex from C-subunit, PHB, Ca²⁺ and polyP.