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This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Nesci, S. (2022). What happens when the mitochondrial H⁺-translocating F₁FO-ATP(hydrol)ase becomes a molecular target of calcium? The pore opens. *BIOCHIMIE*, 198, 92-95 [10.1016/j.biochi.2022.03.012].

Availability:

This version is available at: <https://hdl.handle.net/11585/881174> since: 2022-05-20

Published:

DOI: <http://doi.org/10.1016/j.biochi.2022.03.012>

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Nesci S. Biochimie. 2022; 198: 92-95.

The final published version is available online at:
<https://doi.org/10.1016/j.biochi.2022.03.012>

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What happens when the mitochondrial H⁺-translocating F₁F₀-ATP(hydrol)ase becomes a molecular target of calcium? The pore opens

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Abstract

The F₁F₀-ATPase has Mg²⁺ cofactor as the natural divalent cation to support the bifunctional activity of ATP synthesis and hydrolysis. Different physio(patho)logical conditions permit the molecular interaction of Ca²⁺ with the enzyme and the modification of the biological role. Three distinct binding regions of Ca²⁺ have been localized on the enzyme complex: one in the F₁ catalytic sites and the other two sites in the membrane-embedded domain F₀. In all likelihood, Ca²⁺-activated enzyme most frequently works as an H⁺-translocating F₁F₀-ATP(hydrol)ase with a monofunctional activity that triggers the formation of mitochondrial permeability transition pore (mPTP) phenomenon. The protein(s) component of the mPTP is considered an arcane mystery. However, the F₁F₀-ATPase could reveal the molecular mechanism of pore opening when Ca²⁺ is bound to the enzyme. In this regard, the role of Ca²⁺-dependent function of the F₁F₀-ATPase in the formation of the mPTP is discussed.

Keywords: F₁F₀-ATPase; calcium; permeability transition pore; mitochondria; divalent cations.

1. Introduction

The F₁F₀-ATPase is a unique enzyme in biology that perform the synthesis of ATP with an energy transduction mechanism driven by the proton motive force (Δp) through the inner mitochondrial membrane. Conversely, the F₁F₀-ATPase can work in reverse as an H⁺ pump and the chemical energy of ATP phosphoanhydride bonds is used to energize the membrane. The torque generation of the membrane complex, arising by the coupling activities of the ions translocator F₀ domain and the F₁ catalytic domain, is the universal feature of the energy-transducing membrane. Basically, the Δp conversion to rotational kinetic energy is exploited for the formation of high-energy phosphodiester bonds [1]. Therefore, the Δp -driven torsion of the *c*-ring palisade in the F₀ domain is transmitted via the central stalk to the mechanochemical power of the ($\alpha\beta$)₃-fold symmetric globular structure in the F₁ domain. As result, three ATP molecules are synthesized with a complete revolution of the rotor. The hydrophilic F₁ domain and the hydrophobic F₀ domain perform the chemical reactions of ATP or the H⁺ translocation, respectively. Both domains are functionally coupled by the central stalk rotation while they are structurally connected by a peripheral stalk to prevent idle rotation of F₁ with F₀ [2] (Fig. 1A).

2. The F₁ catalytic sites

The F₁ domain has three catalytic sites alternating with three non-catalytic sites on β and α subunits, respectively at α/β subunit interfaces. The three catalytic sites throughout allosteric cooperation and sequential conformational states are filled with adenine nucleotide (AN), *i.e.* ATP and/or ADP, or empty. The β_{TP} , β_{DP} and β_E conformation, which is dependent on the direction of rotor rotation, adequately represents the “tight”, “loose”, and “open” conformation suggested for the binding change mechanism required by F₁F₀-ATPase to perform ATP synthesis or hydrolysis [3]. Therefore, the “open” conformation is not occupied by AN, whereas the ANs are bound in the “tight” and “loose” conformation of β subunit. Conversely, Mg²⁺ plus ATP to sustain the ATP hydrolysis of β subunit are present in each α subunit [4,5]. However, the recent cryo-EM of ATP synthase has revealed that ADP and Mg²⁺ are also present in each α subunit [4]. In the metal binding pocket of β or α subunit, a P-loop or phosphate-binding loop responsible for binding nucleotide phosphate has the terminal Thr residue (Thr_{P-loop}) directly linked to the divalent cofactor (Fig. 1B). Thr¹⁶³ and Thr¹⁷⁶ are found in the P-loop of β and α subunit, respectively. The divalent metal binding pocket in the catalytic site can be occupied by other divalent cations changing the ATP hydrolysis kinetic parameters (V_{max} and K_m) of the F₁F₀-ATPase [6–8]. The β subunits have different conformational states when occupied by Ca²⁺ as compared with the natural cofactor Mg²⁺. In bacteria, the Thr_{P-loop} mutation reduces Ca²⁺ binding affinity into the catalytic nucleotide-binding sites of the β subunits and decreases Ca²⁺-ATPase activity [9,10]. Consistently, the mutation of the Thr_{P-loop} in the F₁F₀-ATPase increased the Mg²⁺-dependent-ATP hydrolysis, whereas Ca²⁺-activated F₁F₀-ATPase activity was nearly completely prevented [11]. Direct allosteric mechanisms mediated by Ca²⁺ ions were ruled out already in the 1990s [12]. The β subunits behave as low affinity and moderate capacity calcium-binding proteins. Conversely, an equivalent behaviour is not shown by the homologous α subunit and the F₁F₀-ATPase supports the specificity of calcium-binding protein in the catalytic sites [13].

Ca²⁺ has recently been suggested to modulate the mitochondrial F₁F₀-ATPase activity and to play a role in the formation of the mitochondrial permeability transition pore (mPTP) under physio(phato)logical conditions in cells [7,11,14–16]. Indeed, Ca²⁺-activated F₁F₀-ATPase transition, by switching from an energy-conserving enzyme to the energy-dissipating structure, has been identified as the essential event that triggers the mPTP formation [7,17,18]. Ca²⁺ has a higher steric hindrance than Mg²⁺. The binding of Ca²⁺ to the β subunits in the F₁ domain, with its larger ionic radius, can elicit conformational changes in the entire F₁F₀-ATPase. The cofactor binding site involves the known Thr_{P-loop}, which directly interacts with Mg²⁺, and three water molecules used as bridges to β Arg¹⁸⁹, β Glu¹⁹², β Asp²⁵⁶ residues to coordinate Mg²⁺. The six-fold octahedral coordination geometry is completed by Mg²⁺ interaction with two phosphate groups of adenine nucleotide. However, the greater steric hindrance of Ca²⁺ with respect to Mg²⁺, when inserted in the β subunits (Fig. 1B), implies irregular bond distances and angles in the F₁ catalytic sites up to allow eight ligands and a less rigid geometry resulting in irregular bond distances [19,20].

3. The mPTP-opening phenomenon of F₁F₀-ATPase

The mechanism through which the F₁F₀-ATPase forms the mPTP is under intense investigation. Specifically, the ATP hydrolysis sustained by Ca²⁺ could be the potential event in determining the transition to the enzyme of death by mPTP opening [7,18]. The catalytic sites of the F₁F₀-ATPase adopt different conformations when Ca²⁺ or Mg²⁺ are bound and support ATP hydrolysis [4,8]. Ca²⁺-dependent mPTP activity is readily inhibited by Mg²⁺ADP [7,14–16] indicating that the AN-binding sites are critical in the mPTP formation. Thus, a different mechanism of enzyme catalysis with Ca²⁺ might trigger the mPTP. It is interesting to understand that a molecular mechanism in the hydrophilic F₁ domain can influence the mPTP-opening phenomenon in the membrane. A body of literature suggests that OSCP-mediated flexible coupling has broad physiological implications [2]. Among these, the subunit OSCP has N- and C-terminal domains connected by a hinge that

facilitates flexible coupling of AN catalysis and H⁺-translocation with ≈30° back-and-forth rotation of the N-terminal domain with F₁ relative to the C-terminal OSCP domain and peripheral stalk [2]. The flexible hinge of OSCP may make difficult the F₁/peripheral stalk assembly to accommodate larger than normal conformational changes of the β subunits loaded with Ca²⁺. Therefore, the strong distortion of the peripheral stalk encompasses the conformational transmission in the membrane to a “hook apparatus” of a triple transmembrane helix bundle (TTMHB) formed by subunits *b*, *g*, and *e*. The TTMHB can be responsible for the mechanism in the “bent-pull” model of the *c*-ring channel gating from the side facing the intermembrane space [4,21]. The retraction of the *e* subunit, which stems from the destabilization of salt bridges between the TTMHB, pulls the linked lyso-phosphatidylserine (LPS) plug out of the *c*-ring at the intermembrane space. Meanwhile, the enzyme destabilization pulls out phosphatidylserine at the opposite side of the *c*-ring and the mPTP opens [4,18]. The hypothesis highlights the ability of the mitochondrial F₁F₀-ATPase to switch the bi-directionality of the ATP energy transduction in the presence of the natural cofactor Mg²⁺ into the ATP dissipator that forms the mPTP in the presence of Ca²⁺ as cofactor.

4. Unusual Ca²⁺-binding sites of F₀ domain

Noteworthy, dephosphorylated *c* subunit in the presence of threshold Ca²⁺ concentration promotes F₁F₀-ATPase activity. Phosphorylated/dephosphorylated forms of *c* subunits have different hydrophobicity and low phosphorylation potential, *e.g.* dephosphorylated *c* subunits, and could be more effective in stimulating the opening of cyclosporin A-sensitive mPTP. Indeed, the pore with the dephosphorylated *c* subunits has an ion conductance by different orders of magnitude higher and with longer open times than the phosphorylated form [22]. The phosphorylation/dephosphorylation post-translational modification of the *c* subunits changes the conformation of the *c*-ring as well as the interaction between the hairpin structure of the *c* subunits itself allowing Ca²⁺ binding on the N-terminus of *c* subunits (intermembrane space) (Fig. 1C), which may induce a global unfold of the membrane protein. During the hypothesized mechanism of the “bent-pull” model, Ca²⁺-dependent interaction with the N-terminus of *c* subunits might facilitate the LPS plug expulsion from the central hole inside the *c*-ring by hydration of the intermembrane space of the F₀ domain. In the first step of mPTP formation, the retraction of the “hook apparatus” is a crucial event. Accordingly, the *e* subunit removes the LPS from the *c*-ring. Therefore, the LPS displacement from the rotor could be promoted by a solvation process of attraction between water molecules and Ca²⁺ bound to the N-terminus of *c* subunits.

Moreover, calmodulin-binding proteins were found to be associated with mitochondrial membranes and residues 34-65 of the *e* subunit show homologous sequences corresponding to Ca²⁺-dependent tropomyosin-binding regions in troponin T [23] placed immediately after the “hook apparatus” (Fig. 1D). Since the phenylglyoxal-sensitive ARG₉₆ of *g* subunit can open the mPTP [16] likewise the binding of Ca²⁺ on the *e* subunit could rearrange the tight packing of helices in the TTMHB. In particular, the interactions between the conserved GXXXG motifs of *e* and *g* subunit that associate them (Fig. 1D), allow the lipid plug to get out of the *c*-ring hole.

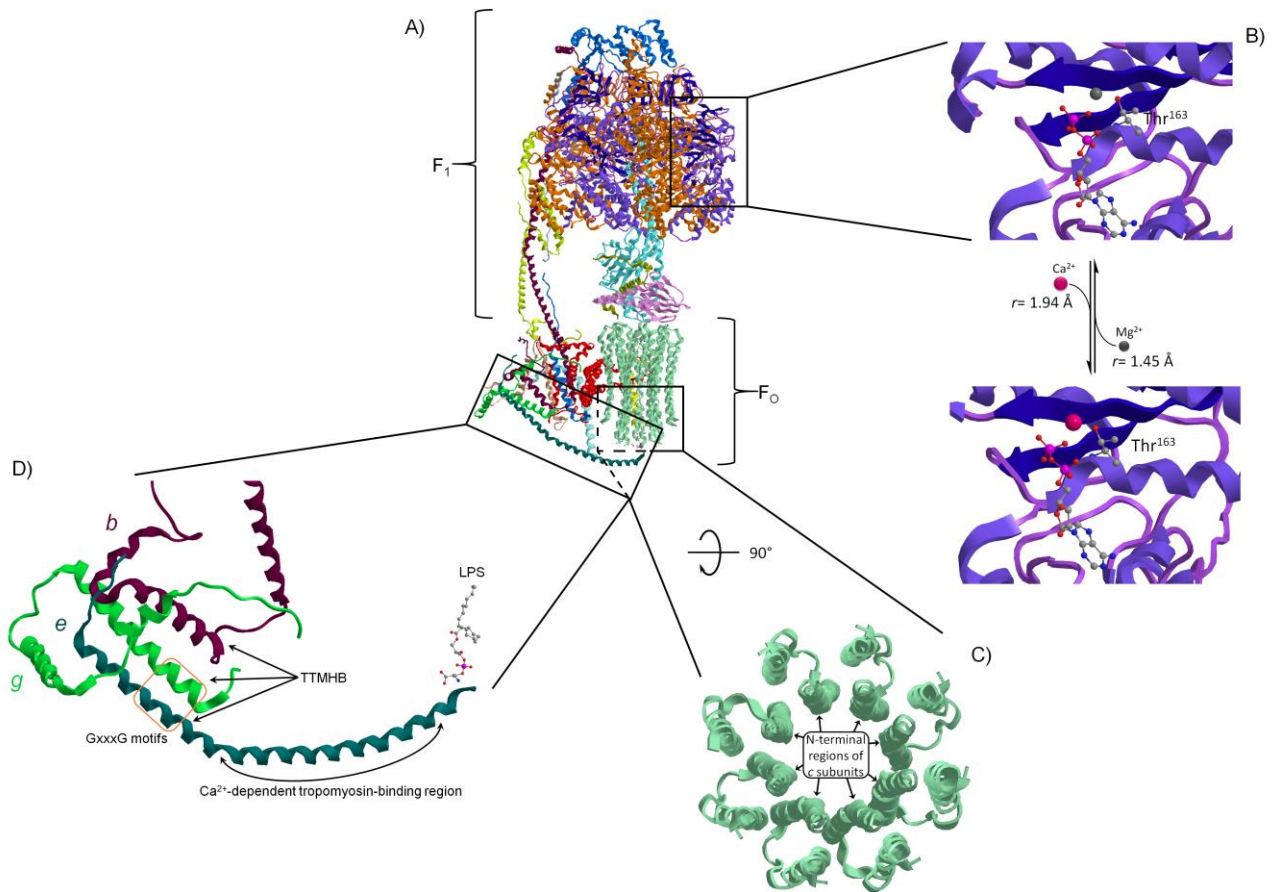
5. Conclusion

To sum up, Ca²⁺ binding to F₁F₀-ATPase triggers a mechanical signal transduction that induces the conformational changes that form the channel bioarchitecture identified as mPTP [24]. The structural knowledge accumulated up to now is strongly consistent with this mechanism.

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Figure

Figure 1. Ca^{2+} binding sites in the mitochondria F_1F_0 -ATPase. A) The F_1F_0 -ATPase monomer is drawn as ribbon representations obtained from modified PDB ID code: 6TT7. B) MgADP or CaADP are bound to the catalytic site. The β_{DP} subunit (violet), drawn as a ribbon representation, is obtained from modified PDB ID code: 6YYO and the βThr^{163} side chain, which directly interacts by electrostatic bonds with the divalent cation, and the ADP molecules are drawn as a ball and stick representation. Mg^{2+} and Ca^{2+} are drawn as a sphere grey and amaranth, respectively. The “ r ” indicates the atomic radius of the divalent cations. C) The c -ring (light green) drawn as ribbon representation obtained from modified PDB ID code 6TT7, is viewed from the intermembrane space side. The N-terminal region of each c subunit is the candidate binding site for Ca^{2+} . D) The “hook apparatus” of the F_0 domain formed by the b , g , and e subunits is obtained from modified PDB ID code: 6TT7. The compact triple transmembrane helix bundle (TTMB) is indicated by arrows. In the red box, the conserved GxxxG motifs of the g and e subunits are shown. Ca^{2+} -dependent molecular interactions placed on the like tropomyosin-binding region protrude into the intermembrane space. Lyso-phosphatidyl-serine (LPS), drawn as a ball and stick model, bind to e subunit by its polar head at the intermembrane space side.