Protein folding and unfolding: proline cis-trans isomerization at the c subunits of F1 F0-ATPase might open a high conductance ion channel

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Abstract

The c subunits, which constitute the c-ring apparatus of the F1 F0-ATPase, could be the main components of the mitochondrial permeability transition pore (mPTP). The well-known modulator of the mPTP formation and opening is the cyclophilin D (CyPD), a peptidyl-prolyl cis-trans isomerase. On the loop, which connects the two hairpin α-helix of c subunit, is present the unique proline residue (Pro40) that could be a biological target of CyPD. Indeed, the proline cis-trans isomerization might provide the switch that interconverts the open/closed states of the pore by pulling out the c-ring lipid plug.

Keywords: F1 F0-ATPase; mitochondria; permeability transition pore; c-ring; cis-trans isomerization.

The c subunits of F1 F0-ATPase are multifunctional proteins whose energy transduction features cover the transmembrane H+ translocation, whereas stoichiometry determines the species-specific bioenergetic cost of ATP [1]. The helical hairpin structure of the c subunit has the N- and C-terminal faced on the cytoplasm side of the inner mitochondrial membrane (IMM), whereas the amino acids that form the loop region (c-loop) and connect the two transmembrane α-helix are faced on the matrix side of IMM. Moreover, the loops of each c subunit are joined to the foot of the hydrophilic F1 portion of the enzyme. The annular arrangement of c subunits oligomer, which constitutes the so-called c-ring, has two concentric circles. The inner circle is composed of the N-terminal helix, whereas the outer circle is by the C-terminal helix of c subunits (Fig. 1). These packed hairpins form a sort of hourglass, seen laterally from the membrane side,
whose in the middle of the membrane the concavity of the c-ring hosts on the C-terminal helix the H\textsuperscript{+} binding site, which is represented with a conserved Glu\textsubscript{58} residue in the mitochondrial F\textsubscript{1}F\textsubscript{0}-ATPase [2]. In addition to this, to ensure the rotation of the rotor driven by protonmotive force (\(\Delta p\)) in the ATP synthesis mode or dissipation of \(\Delta p\) during the ATP hydrolysis, the carboxylic side chains of Glu\textsubscript{58} switch from deprotonated to protonate conformation during the ion translocation. Indeed, the H\textsuperscript{+} binding site exposed to a hydrophilic environment within the half-channels of a subunit is oriented in an outward-facing open conformation (the carboxylic group adopts the unlooked conformation), whereas into the hydrophobic environment inside the IMM during the rotor rotation it re-orients to an inward-faced closed conformation (H\textsuperscript{+} locked conformation) with a favoured energy state to enter the IMM [3].

Importantly, the c-ring is filled with two different phospholipids at the two opposite sides of its cavity (Fig. 1). At the matrix side, phosphatidylserine (PS) is anchored by electrostatic coordination to the positive charge of Arg\textsubscript{38} of subunits. On the cytoplasm side of IMM, Lys\textsubscript{71} of c subunit by ionic interaction binds a lyso-phosphatidylserine (L-PS) [4]. The positive charge residue of the Arg\textsubscript{38} side chain of each c subunit by facing the cavity of the c-ring at the matrix side coordinate the negatively charged PS. The Arg\textsubscript{38} residues of c subunits are placed at the end of the C-terminal helix and form, together with Asn\textsubscript{39} residues, the positive collar of c-loop at the boundary of the PS polar heads. The PS inserted in the hole of the c-ring with the two acyl chains fill all the space. Moreover, the tight fit of the double-chained matrix-side lipid into the c-ring establishes also hydrophobic interaction with a glycine zipper (G\textsubscript{20}xG\textsubscript{22}xG\textsubscript{24}xG\textsubscript{26}) and this suggests that the PS rotate with the c-ring. The Gly\textsubscript{26}-Glu\textsubscript{26} mutation within the glycine-rich region of c subunits is responsible for the mitochondrial permeability transition pore (mPTP)-mediated hypoxia/reoxygenation cell death in cardiomyocytes [5] by missing the interactions of the acyl chains with the c subunits. Indeed, by the addition of negatively charged residue in the helix structure, it could favour the instability of phosphatidylserine in the c-ring and its expulsion counted in the possible mechanism of the mPTP phenomenon [6].

The pore forms from the c-ring

Apart from being the main producer of ATP in mitochondria, the F\textsubscript{1}F\textsubscript{0}-ATPase has other crucial roles in energy-related homeostases, such as assisting mitochondrial cristae curvature and the cell death regulation via the mPTP [7]. Switching the energy transduction system from the energy-saving to the energy-dissipating mode is a new feature of the F\textsubscript{1}F\textsubscript{0}-ATPase [8,9]. The c subunits, which constitutes the c-ring, are considered the main components of the mPTP [4,10,11]. According to the new “bent-pull” model [12], the Ca\textsuperscript{2+}-activated F\textsubscript{1}F\textsubscript{0}-ATP(hydrol)ase activity generates a force transmitted from the F\textsubscript{1} catalytic subunit to the membrane-embedded subunit of the F\textsubscript{0} domain. On the cytoplasm side of IMM, the lipid plug of L-PS is pulled out of the c-ring by the movement of the c subunit. On the other side of the c-ring cavity, the PS coordinate with the c subunit fills the hole and block the pore. The water molecules coming into the c-ring exert a thrust force on the PS [4]. The enlargement of the c-ring collapsed on solubilization or a conformational change of structure could loosen the lipid interaction with the c subunits. The consequent modification of the c-ring can also alter the interaction with the foot of the central stalk by favouring the detachment of the F\textsubscript{1} from the F\textsubscript{0} and the PS is pushed out and creates a pore through the c-ring [4,9].

The hypothesized effect of CyPD on the (un)folding of the c subunits

Cyclophilin D (CyPD) is a mitochondrial chaperone protein identified as a peptidyl-prolyl, cis-trans isomerase (PPIase), which might be involved in mitochondrial protein folding, but there are no results on the presence of this activity [13]. Moreover, CyPD is a modulator of the mPTP formation and opening [14]. CyPD can bind the F\textsubscript{1}F\textsubscript{0}-ATPase and has been suggested that the OSCP subunit of the peripheral stalk of the F\textsubscript{1}F\textsubscript{0}-ATPase is its direct interactor in the enzyme complex [15]. The interdomain hinge of the OSCP subunit facilitates flexible coupling of the rotation to conformational changes of the catalytic subunits and makes this subunit an apposite point for the regulation of ATP synthesis [16]. The hinge flexibility of the N-terminal OSCP domain linked to the F\textsubscript{1} sector relative to the C-terminal OSCP domain joined to the peripheral stalk is blocked by CyPD binding (Fig 2). This molecular event reduces the elastic movement of F\textsubscript{1} with respect to the rotor permitting the signal transmission to the membrane-embedded F\textsubscript{0} sector where
the pore opens [17,18].

On the c-loop that connects the two hairpin α-helix of the c subunit is present the unique proline residue (Pro 40) which could be a biological target of CyPD in the hydrophilic space between the c-ring and the foot of the central stalk (Fig. 2). Indeed, in cells overexpressing the c subunit, the mPTP is inhibited by cyclosporin A (CsA) the inhibitor of CyPD [11]. The CsA can inhibit the mPTP opening at an early stage but not at later ones. The c subunits could also form an ion channel by assembling into oligomers in a β-sheet conformation with a similar mechanism to some other amyloidogenic peptides that form a β-sheet oligomeric pore [19]. Recently, it has been suggested that CyPD-c subunit interactions help the formation of higher-order oligomers, but is not required for pore activity by highlighting the folding activity in the mPTP formation [20].

The cis-trans isomerization of the Pro might provide the switch that interconverts the pore open/closed states by pulling out the c-ring lipid plug. The Pro is the only amino acid with cis-trans-isomerization in the peptide bond involving its imino nitrogen (Fig. 3). Peptide bonds between amino acids residues are preferentially in the trans configuration, whereas the cis configuration occurs at β turns involving the Pro isomer. However, the Pro forms cis peptide bonds at a frequency higher than any other naturally occurring amino acid. The switch from trans to cis is a biological structural mechanism exploited in the channel opening [21]. The Pro located at the apex of the loop between two transmembrane helices can link binding to gating through a cis-trans isomerization of the protein backbone. The hypothesis is that the natural trans configuration of the Pro 40 allows the closed state of the pore. Conversely, by converting the Pro 40 to the cis conformation and treating the N-terminal helix of the c subunit as a rigid body, the c-ring enlarge the hole (Fig. 3) and the environment hydrated by incoming water molecules pulls the lipid plug out and obtains the open structure of the mPTP. Therefore, the trans-cis isomerization at Pro 40 could function as a hinge for the movement of α-helix during gating and explain the reported results that c subunit alone without other parts of F1FO-ATPase is sufficient to induce the mPTP formation [11].

Conclusion

The mitochondrial protein folding could be affected through the CyPD, but it might also achieve a scaffolding function, as it binds to several proteins in the mitochondrial matrix and the IMM [13]. The rotamase activity of PPIases increases the proline isomerization by up to 260 fold with an intramolecular hydrogen bond to the prolyl amide nitrogen [22]. If the CyPD has conserved structural features that facilitate cis-trans isomerization, it is a kinetically viable candidate for the gating switch. On balance, this change at a crucial pivot point on the c-loop reorients the transmembrane helices of c-subunits forming the pore. The cis-trans isomerization of this single Pro 40 could provide the switch that interconverts the open and closed states of the mPTP by CyPD-dependent manner through the c-ring.

Conflict of Interest

None

References


Figure 1. The c -ring structure of F1FO-ATPase is obtained from modified PDB ID code: 6TT7. On the right, the structure of mammalian F1FO-ATPase with the c -ring highlighted in purple. On the left, the spatial arrangement of the c subunits in cartoons mode. In the middle, the c -ring structure is viewed laterally. The red box highlights the region of the glycine zipper present on the N-terminal helix. On the top, the c -ring is viewed from the matrix. In the hole of the c -ring, the phosphatidylserine (as a ball-and-stick model) is specifically coordinated with the Arg38 residues drawn as a stick on the N-terminal helix of each c subunit. ThePro40 is illustrated as a space-filling model on the c -loop. The c -ring is viewed from the cytoplasm side of the IMM (lower panel). The lyso-phosphatidylserine (L-PS) as a ball-and-stick model is depicted in the middle of the c -ring.

Figure 2. The CyPD binding sites of F1FO-ATPase. On the left the hydrophilic portion of F1FO-ATPase is obtained from modified PDB ID code: 6TT7. The interdomain hinge of the OSCP subunit is coloured red. On the right the rotor (the foot of central stalk: γ, δ, and ε subunit) and the c -ring obtained from modified PDB ID code: 6TT7. The Pro40 and the phosphatidylserine (PS) are illustrated as a ball-and-stick model. The discs highlight the sites of CYPD (PDB ID code: 3QYU) interactions.

Figure 3 Proline isomers of c subunits. The immino nitrogen of proline (red structure) is involved in the torsional peptide bond (dashed blu) of the reversible trans and cis isomers. Two opposite c subunits (c ‘ and c ”) of the c -ring with the Pro40 in trans configuration (on the left) and cis configuration (on the right) show the difference of the hole (dotted shapes).