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Hypothesis

Regulation of the F_0F_1-ATP synthase: The conformation of subunit ε might be determined by directionality of subunit γ rotation

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Abstract **F_0F_1-ATP synthase couples ATP synthesis/hydrolysis with transmembrane proton transport. The catalytic mechanism involves rotation of the $\gamma\varepsilon c_{\sim 10}$-subunits complex relative to the rest of the enzyme.**

In the absence of protonmotive force the enzyme is inactivated by the tight binding of MgADP. Subunit ε also modulates the activity: its conformation can change from a contracted to extended form with C-terminus stretched towards F_1. The latter form ihnibits ATP hydrolysis (but not synthesis).

We propose that the directionality of the coiled-coil subunit γ rotation determines whether subunit ε is in contracted or extended form. Block of rotation by MgADP presumably induces the extended conformation of subunit ε. This conformation might serve as a safety lock, stabilizing the ADP-inhibited state upon de-energization and preventing spontaneous re-activation and wasteful ATP hydrolysis. The hypothesis merges the known regulatory effects of ADP, protonmotive force and conformational changes of subunit ε into a consistent picture.

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1. Introduction

F_0F_1-ATP synthase interconverts two major "energy currencies" of a living cell: the transmembrane electrochemical potential difference of protons (or Na^+ in some organisms) and ATP. The enzyme is composed of two portions: membrane-embedded F_0-portion (in the simplest bacterial enzyme a complex of three types of subunits in stoichiometry $a_1b_2c_{\sim 10}$) and hydrophilic catalytic F_1-portion (in bacteria a complex of five types of subunits in stoichiometry $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1$; see Fig. 1 for a cartoon representation of the enzyme). A unique feature of the enzyme is its rotary catalytic mechanism [1–3]. Proton transport through F_0 driven by protonmotive force (pmf) induces rotation of the ring-shaped c-subunits oligomer relative to the peripherally located a_1b_2. Subunits γ and ε are bound to the c-ring and therefore rotate relative to the $\alpha_3\beta_3\delta_1$ complex that is attached to the elongated b-subunit dimer. Rotation of subunit γ inside the $\alpha_3\beta_3$-hexamer induces conformational transitions that result in ATP synthesis.

When pmf drops below the thermodynamic threshold of ATP synthesis, the reaction is reversed and the enzyme operates as an H^+-pumping ATPase (see [4–7] for reviews on the catalytic mechanism).

A common regulatory mechanism found in ATP synthases from bacteria, chloroplasts and mitochondria is so-called "ADP inhibition". When MgADP without phosphate is bound at the high affinity catalytic site, the enzyme is inactivated in terms of ATP hydrolysis [8–13]. As demonstrated for the chloroplast and bacterial enzymes, upon membrane energization the tightly bound ADP is released from F_1 [14–16]. This might be the reason for the marked increase in the ATPase activity after energization (so-called pmf-activation) [17–21]. In bacterial ATP synthase subsequent slow deactivation of ATP hydrolysis occurs after pmf dissipation; it is accelerated by ADP and slowed down by phosphate, indicating that the enzyme shifts back to the "ADP-inhibited" state [18,21]. It is demonstrated on the single molecule level in bacterial enzyme that such inhibition results in long (≈30 s) pauses in the ATP driven rotation of subunit γ [22]. Further experiments indicate that the re-activation can be achieved by forced rotation of subunit γ from the "ADP-inhibited" position by 40° in the hydrolysis direction; preliminary data indicate that rotation by 160° in the synthesis direction has the same effect [23]. Without forced rotation the spontaneous re-activation from the "ADP-inhibited" state might be induced by thermal rotational fluctuations of subunit γ, and is completely blocked if the angular position of subunit γ is fixed by external force [23].

It should be noted that free phosphate is playing and important role in counteracting the ADP inhibition. Under de-energized conditions the concentrations of phosphate required to relieve the ADP inhibition are rather high (5 mM for the mitochondrial [11] and 20 mM for the bacterial enzyme [24]). However, the affinity of ATP synthase for phosphate dramatically increases in the presence of pmf [25–27]. This increase is considered to be one of the main energy-requiring steps in ATP synthesis. It is proposed that such high affinity to phosphate might be necessary to prevent the competitive inhibition by ATP during ATP synthesis (see [5,28] and the references therein). The increase in the affinity for phosphate is expected to diminish ADP inhibition when sufficiently high pmf is present.

Another mechanism of ATP synthase regulation (demonstrated in bacteria and chloroplasts) is associated with subunit

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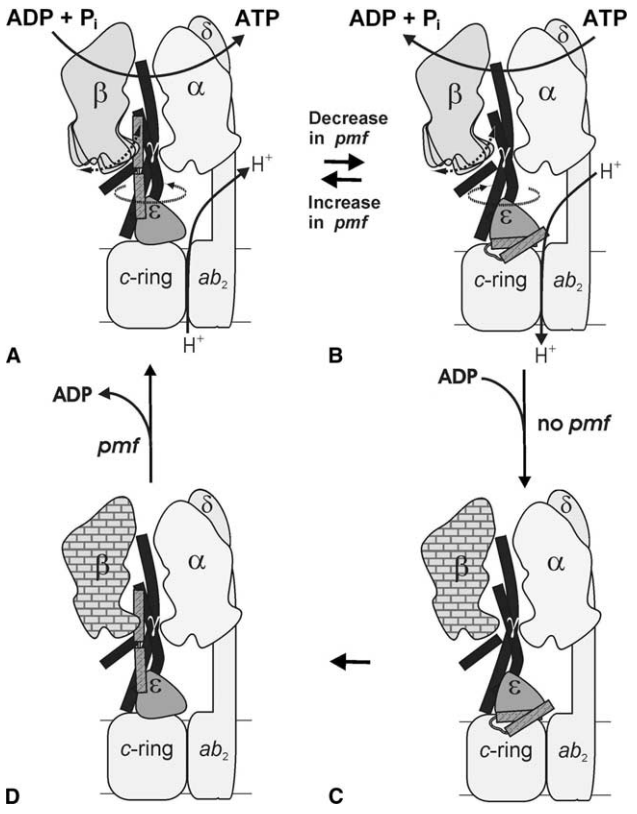

Fig. 1. A tentative scheme of the regulatory conformational transitions in ATP synthase. (A) Active state, ATP synthesis. The two C-terminal α-helices of subunit ε (striped) are in the extended or fully extended state (fully extended state is drawn in the cartoon) (B) Active state, ATP hydrolysis coupled to proton pumping. Subunit ε is in the contracted state. (C) Hypothetical transition state; rotation of subunit γ is blocked due to the binding of ADP without phosphate at the high affinity catalytic site, but the transition of subunit ε C-terminus into the extended or fully extended state has not yet occurred. (D) "ADP-inhibited" state; rotation of subunit γ is blocked by the tight binding of ADP and by interactions of subunit ε C-terminus with β DELSEED fragment.

ε. [1] The inhibitory effect of this subunit on the ATPase activity is well established [29–34]. It should be noted that inhibition by subunit ε is clearly distinct from the MgADP inhibition described above, because the latter is also observed on the $\alpha_3\beta_3\gamma$ complex without ε [22,23,35,36].

It is well known that membrane energization, ATP, ADP, and phosphate induce conformational changes in the ε subunit of chloroplast and bacterial ATP synthase [37–41]. The hint on the molecular details of these conformational transitions have come from two crystal structures: the γε-complex from *Escherichia coli* [42] and the bovine mitochondrial F_1-portion [43]. In the former structure the two C-terminal α-helices of subunit ε are apart and are extended towards F_1 (extended conformation), while the latter structure indicates that these two C-terminal α-helices form a hairpin structure close to F_0 (contracted conformation).

The cross-linking experiments on ATP synthase from *E. coli* revealed that the extended conformation of subunit ε inhibits ATPase activity but not ATP synthesis. In the contracted conformation the enzyme performs well both in synthesis and hydrolysis [44]. It is also demonstrated that in bacterial enzyme the inhibitory effect is due to the electrostatic interactions of the basic C-terminal amino acid residues of subunit ε with the acidic DELSEED fragment of β-subunit [45]. These findings are further supported by the elimination of the inhibitory effect upon the deletion of subunit ε C-terminal α-helices observed both in bacterial [46] and in chloroplast enzyme [47].

Subsequent studies on thermophilic *Bacillus PS3* have revealed that the two C-terminal α-helices of subunit ε can extend even further, penetrating into the $\alpha_3\beta_3$-hexamer to the very N-terminus of γ-subunit. Like the extended conformation, this "fully extended" conformation suppressed ATP hydrolysis without significant effect on ATP synthesis [48]. Recent experimental data on the chloroplast ATP synthase demonstrate that the truncation of 8–20 N-terminal amino acid residues of subunit γ markedly diminishes inhibition of ATPase activity by the ε-subunit [49], indicating that interaction of ε C-terminus and γ N-terminus in the fully extended might also be important for the inhibition.

It is also confirmed that in bacterial ATP synthase subunit ε adopts the contracted conformation in the presence of ATP, while ADP induced the fully extended conformation [48]. Surprisingly, pmf also induces the fully extended conformation that is inactive in terms of ATP hydrolysis [48]. The latter finding is in a seeming contradiction with the experimental evidence on the activation of ATP hydrolysis by pmf [17–21]. The hypothesis presented below provides a solution for this controversy and suggests how the two regulatory mechanisms described above might be interconnected.

2. Hypothesis

The hypothetical scheme of subunit ε regulatory conformational transitions is presented in Fig. 1. Panel A illustrates the enzyme in the "normal" ATP synthesis mode in the presence of high pmf. Most probably in this mode the C-terminus of subunit ε is in the extended or fully extended state (this assumption is based on the experimental data confirming that pmf induces the latter conformation [48]). When pmf decreases below the thermodynamic threshold for ATP synthesis, ATP-driven proton pumping occurs, and the direction of subunit γ rotation reverses. We postulate that the rotation of subunit γ in ATP hydrolysis direction is inducing the conformational transition of subunit ε from the extended or fully extended to the contracted state. The energy input for this transition comes from ATP-driven rotation; we suggest that this rotation is critically important to maintain the contracted conformation of ε. With subunit ε in the contracted state, ATP synthase generates pmf at the expense of ATP hydrolysis (Fig. 1B). When the membrane is re-energized, the enzyme switches back to synthesis mode; the rotation of subunit γ is reversed and the C-terminus of subunit ε moves back towards F_1 (Fig. 1A).

If the rate of pmf dissipation exceeds the rate of ATP-driven proton pumping, the membrane is de-energized, and the affinity for phosphate decreases. This favours the "ADP-inhibited" state of the enzyme: as soon as MgADP binds to (or fails to dissociate from) one of the catalytic sites, the rotation of subunit γ stops [22] (Fig. 1C). As postulated above, the contracted conformation of subunit ε is maintained only when subunit γ

[1] To our knowledge, no experimental evidence is reported so far on the regulatory role of mitochondrial ATP synthase subunit δ, which is homologous to bacterial/chloroplasts ε-subunit.

rotates in the hydrolysis direction, so the transition to extended/fully extended state occurs (Fig. 1D).

The rationale behind this postulate is that the extended state of subunit ε might be thermodynamically favourable due to the electrostatic interactions between the C-terminus of subunit ε and the β DELSEED segment [45]. It is probable that these electrostatic interactions significantly impede the stochastic rotational fluctuations of subunit γ, stabilizing the ''ADP-inhibited'' state and preventing ADP release and spontaneous re-activation of ATP hydrolysis. We suggest that subunit ε functions as a ''safety switch'' that fixes the ADP-inhibited state under de-energized conditions, but is readily relieved upon energization. In the latter case the pmf-driven rotation of γε-complex distorts the interaction of subunit ε C-terminus and the β DELSEED fragment, as well as expels the tightly bound inhibitory MgADP from F_1.

Indirect evidence for this hypothesis is provided by the experiments in *E. coli* on γMet23Lys mutant, where an extra salt bridge between the γLys23 and βGlu381 (the first glutamate in the β DELSEED fragment) is formed [50]. It is noteworthy that the F_1-portion of the mutant enzyme has lost the ability to relieve the subunit ε inhibition upon binding to F_0 [51], behaving as if subunit ε was permanently in the extended/fully extended conformation. It is most probable that the additional bond between γ and β in γMet23Lys mutant plays the same role as the interactions of β DELSEED with basic residues at the C-terminus of subunit ε, hindering subunit γ rotation and stabilizing the ''ADP-inhibited'' state.

It is unclear how exactly the direction of subunit γ rotation may induce the transitions of subunit ε. However, it is tempting to suggest that the chiral coiled-coil region of subunit γ might act as a ''molecular Archimedes screw'' squeezing the elongated mobile C-terminus of subunit ε out of the $\alpha_3\beta_3$-hexamer upon rotation in one direction and letting it slide back upon the reversal or block of rotation.

It should be noted that for the sake of simplicity and to avoid excessive speculation, the hypothesis ignores several other ATP synthase regulatory features, such as relieve of ADP inhibition upon binding of ATP to the non-catalytic sites [52–55] or direct interactions of subunit ε with ATP [56].

It should also be noted that the regulatory mechanism involving the ''up-down'' conformational transitions of subunit ε in the bacterial and chloroplast ATP synthase might be absent in the mitochondrial enzyme. According to the structure of bovine mitochondrial F_1-portion [43], an additional small protein might prevent the conformational transitions, fixing the active contracted state of the mitochondrial homologue of bacterial ε [2] [44]. Further experiments are necessary to clarify this issue.

3. The probable physiological role of the ε-subunit transitions

In a bacterial cell the maintenance of the transmembrane electrochemical proton potential is as important as the maintenance of the high intracellular ATP level. Pmf is necessary for ion transport, motility, protein export and other physiological functions. Reversibility of the ATP synthase provide a powerful tool to maintain pmf at physiological level using ATP produced, e.g., by fermentation or similar processes. However, distinguishing between a temporary decrease in pmf due to low respiratory substrate concentration (or low light intensity in case of photosynthetic bacteria), and complete de-energization (e.g., due to a damaged coupling membrane) could be critical, because in the latter case the active enzyme would just waste the whole ATP pool of the cell. The hypothesis proposed here provides an efficient regulatory scheme to distinguish between these two cases. In case of mild de-energization subunit ε is adopting the contracted conformation and ATP synthase maintains pmf by proton pumping at the expense of ATP hydrolysis. In case of severe de-energization the enzyme adopts ''ADP-inhibited'' conformation and ceases to hydrolyse ATP. Subsequent transition of subunit ε to the extended/fully extended provides a ''safety lock'' that stabilizes the ''ADP-inhibited'' state of the enzyme. Such stabilization might also be important for prompt inhibition of ATP hydrolysis upon accidental removal of the F_1-portion from F_0.

[2] Unfortunately, historically the homologue of bacterial ε is called subunit δ in the mitochondrial enzyme, while the additional small protein (absent in bacterial ATP synthase) is called subunit ε.

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