ATP synthesis catalyzed by the mitochondrial F_1-F_0 ATP synthase is not a reversal of its ATPase activity

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Abstract The ADP(Mg^{2+})-deactivated oligomycin-sensitive F_1-F_0 ATPase of coupled submitochondrial particles treated with the stoichiometric amount of oligomycin was studied to test whether ATP synthesis and hydrolysis proceed in either direction through the same intermediates. The initial rates of ATP hydrolysis, oxidative phosphorylation, ATP-dependent, succinate-supported NAD^+ reduction, and ATP-induced Δρ_{intr} generation were measured using deactivated ATPase trapped by azide [Biochem. J. (1982) 202, 15–23]. Three ATP consuming reactions were strongly inhibited when azide was present in the assay mixtures, whereas ATP synthesis was not altered by azide. The unidirectional effect of azide is not consistent with three alternating binding sites mechanism operating in ATP synthesis and support our hypothesis on the existence of nucleotide(Mg^{2+})-controlled 'synthase' and 'hydrolase' states of the mitochondrial F_1-F_0 ATPase.

Key words: F_1-F_0 ATP synthase; Oxidative phosphorylation; Nucleotide binding site

1. Introduction

The F_1-F_0 ATPase/synthase catalyzes Δρ_{intr}-driven ATP synthesis or Δρ_{intr}-generating ATP hydrolysis in the coupling membranes of mitochondria, bacteria and chloroplasts. A peripheral part of (F_0) is composed of five different polypeptides in the stoichiometry α_1β_1γ_1δ_ε [1] and capable of rapid uncoupled ATP hydrolysis when detached from the membrane embedded F_0 [2]. Three out of six nucleotides bound at αβ subunits of F_0 are rapidly exchangeable with medium ATP or ADP [3,4] indicating that at least three sites may be involved in the catalytic turnovers during ATP hydrolysis/synthesis. F_0 component contains a proton conducting pathway [2] and operating together F_1-F_0 provides Δρ_{intr}-consuming ATP synthesis or Δρ_{intr}-generating ATP hydrolysis depending on physiological (in vivo) or experimental (in vitro) conditions. Several hypotheses on the molecular mechanism of the reaction with participation of three equipotential cooperative nucleotide-binding sites [5,6], two cooperative sites [7] and a single catalytic site [8] have been advanced and extensively discussed [5,6,9].

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Abbreviations: HEPES, N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid; Oxonol VI, 6-[3-(propyl-5-oxoisoxazol-4-yl)pentamethine oxonol.

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SSDI 0014-5793(95)00487-4
3. Results

The time-course of ATP hydrolysis catalyzed by F₁ or F₁-F₀ ATPase in the presence or absence of ATP regenerating system shows either lag or burst depending on the enzyme preconditioning [13]. When submitochondrial particles are preincubated with phosphoenol-pyruvate and pyruvate kinase [13], or ATP [24] or EDTA [14] their initial ATPase activity appears as azide-insensitive burst followed by the slower, azide-sensitive rate [15]. In contrast, when the enzyme is preincubated with stoichiometric amount of ADP in the presence of Mg²⁺ the ATP hydrolysis is completely blocked and the reaction is slowly accelerated in the ATP concentration-dependent fashion. This reactivation does not proceed if azide is present in the assay medium and ATPase remains inhibited for quite a long time [15]. It seemed to be of great interest to find how these active/inactive transitions of F₁-F₀ ATPase correlate with the ability of the enzyme to catalyze other ATP-dependent reactions and, most importantly, oxidative phosphorylation. Fig. 1 shows the time course of ATP hydrolysis and synthesis by Pₐ-activated and ADP(Mg²⁺)-deactivated, azide-trapped (B) F₁-F₀ ATPase. The reactions were initiated by the addition of submitochondrial particles (SMP; 0.1 mg) to 2 ml of the standard reaction mixture containing either 1 mM ATP (ATP hydrolysis) or 1 mM ADP (ATP synthesis). Other components of the reaction mixture and pretreatment of active (SMPₐ) and deactivated (SMPₐ) are described in section 2. 100 μM sodium azide was present in the assay mixtures when the reactions catalyzed by deactivated SMP were measured (B). Gramicidin D (G; 0.2 μg) was added where indicated. Figures on the curves indicate the rates expressed in mmol of ATP hydrolyzed or formed per min per mg of protein.

![Fig. 1. Time-course of ATP hydrolysis (upper curves) and synthesis (lower curves) catalyzed by the coupled active (A) and ADP(Mg²⁺)-deactivated, azide-trapped (B) F₁-F₀ ATPase. The reactions were initiated by the addition of submitochondrial particles (SMP; 0.1 mg) to 2 ml of the standard reaction mixture containing either 1 mM ATP (ATP hydrolysis) or 1 mM ADP (ATP synthesis). Other components of the reaction mixture and pretreatment of active (SMPₐ) and deactivated (SMPₐ) are described in section 2. 100 μM sodium azide was present in the assay mixtures when the reactions catalyzed by deactivated SMP were measured (B). Gramicidin D (G; 0.2 μg) was added where indicated. Figures on the curves indicate the rates expressed in mmol of ATP hydrolyzed or formed per min per mg of protein.](image-url)
reactions were initiated by the ‘deactivated’ SMP. The ATP-induced $\Delta \mu_{H^+}$-generator as seen from the response of the membrane potential-sensitive probe [27] was also inhibited by azide when particles with ‘deactivated’ F$_1$-F$_0$ ATPase were used (Fig. 3).

4. Discussion

Most of the present-day ideas concerning the mechanism of oxidative phosphorylation are based on the structural and kinetic studies of purified F$_1$'s or preparations of F$_1$-F$_0$ which are incapable of net ATP synthesis. Numerous reports on the kinetics of ATP hydrolysis by F$_1$ or F$_1$-F$_0$ preparations different degree of resolution have appeared in the literature but advanced studies on the kinetics of ATP synthesis have been relatively rare [28–30] although it has been claimed that oxidative phosphorylation is much less sensitive to azide than ATPase [31,32]. In fact, we were not aware of any reports where the oxidative phosphorylation is much less sensitive to azide than ATPase (Fig. 3).

Both ATP hydrolysis and NAD$^+$ reduction were more than 95% sensitive to oligomycin (112 nM). The reverse electron transfer was completely prevented by uncoupler (not shown). The final concentrations were: succinate (S) 16 mM; ATP 1 mM; MgCl$_2$ 5 mM; gramicidin D (G) 0.2 mg; oligomycin (Oligo) 12 nM.

Operating during ATP synthesis. Several reports in the literature contradict equipotentiality of the nucleotide-binding sites on F$_1$-F$_0$ type ATP synthases. The central issue of this report concerns the questionable identity of the molecular events during ATP synthesis and hydrolysis by F$_1$-F$_0$ ATPase/synthase. It worth noting that F$_1$-ATPase protein inhibitor (IF$_1$) which is an intrinsic part of the mammalian ATP synthase complex also known to be a ‘unidirectional’ inhibitor of ATP hydrolysis [39]. Although $\Delta \mu_{H^+}$-dependence of F$_1$-F$_0$-IF$_1$ interaction have been proposed to explain the lack of inhibitory effect of IF$_1$ on oxidative phosphorylation [40], such explanation is hard to reconcile with our more recently reported data [41]. In summary, we would like to emphasize that the difference in the catalytic step-by-step mechanism of ATP synthesis and hydrolysis may be a general phenomenon for F$_1$-F$_0$ type ATP synthases.

Acknowledgements: This work was supported by Russian Foundation for Fundamental Research (Grant 93-04-20214) and International Science Foundation (Grant MR 2000). We thank Dr. Elena Maklashina and Mr. Mikhail Galkin for their kind help during this work.

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