Activation of a complex of ATPase with the natural protein inhibitor in submitochondrial particles

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Almost all ATPase molecules in submitochondrial particles, isolated from beef heart mitochondria in the presence of MgATP, are in an inactive complex with the natural protein inhibitor (IF_1). In de-energized particles at high ionic strength a slow and irreversible ATPase activation is found to occur due to a dissociation of the enzyme-inhibitor complex. The pH-dependence of this process points out that deprotonation of IF_1 molecule is an essential step in the dissociation of the complex. Zn^{2+} sharply accelerates ATPase activation, probably via binding with the deprotonated form of IF_1. ATPase activation is completely prevented by MgATP, indicating the formation of a transient enzyme-inhibitor complex retaining ATPase activity

Submitochondrial particle; H^+-ATPase; Protein inhibitor

1. INTRODUCTION

The natural protein inhibitor of ATPase is essential for regulation of the activity of the enzyme in mitochondria. This small protein (M_r 9.5 kDa) binds with a catalytic component of ATPase complex – F_1-ATPase – and blocks ATP hydrolysis, but has no effect on a stationary ATP synthesis (for review see [1]). The major physiological purpose of such regulation probably consists of a prevention of futile ATP hydrolysis in de-energized mitochondria [2]. The inhibitor (IF_1) suppresses ATPase only in the presence of ATP and its activity is enhanced at low pH [3]. A target for IF_1 action is an unidentified transient complex, E*AdN [1,4]. The pH-dependence of the inhibition is determined by a conformational rearrangement coupled to a protonation of the histidine residue in the IF_1 molecule [5,6].

Insensitivity of stationary oxidative phosphorylation to IF_1 is caused by a dissociation of the E^*IF_1 complex under energization [1]. The mechanism of this effect is still obscure. In the present work we studied the effect of pH and MgATP on the enzyme-inhibitor complex activation in de-energized submitochondrial particles (SMP).

2. MATERIALS AND METHODS

SMP from beef heart mitochondria were isolated in the presence of 1 mM MgATP according to [7]. S-SMP were prepared as in [8]. Standard incubation medium contained 250 mM sucrose, 50 mM K_2SO_4, 2 μM CCCP, 1 mM EDTA, 10 mM Tris (pH 7.75–9.2), or 10 mM Hepes (pH 6.7–7.45), or 10 mM Mes (pH 6.0). In some experiments, 1 mM glucose, 0.5 mM $MgSO_4$ and hexokinase (7.6 U/ml) were added and EDTA was excluded from the incubation medium. A contribution of hexokinase reaction, via traces of glucose and hexokinase getting into the spectrophotometric cuvette, to ADP formation was evaluated using oligomycin, an inhibitor of F_0F_1-ATPase. The oligomycin-insensitive fraction did not exceed 10% of the total activity. Incubation was carried out at 37°C.

The following medium was used to measure ATPase activity: 250 mM sucrose, 10 mM Tris, 10 mM KCl, 1 mM phospho*enol*pyruvate, 0.3 mM NADH, 50 μM EDTA, 3 mM $MgSO_4$, 2 mM ATP, pyruvate kinase (5 U/ml), lactate dehydrogenase (5 U/ml), 2 μM CCCP, 2 μM rotenone, pH 8.0. This medium was preincubated with myokinase (1 U/ml) to convert AMP present in the chemicals and to prevent the kinase reaction during the measurements.

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3. RESULTS

3.1. *The pH-dependence of ATPase activation in SMP*

ATPase activity of SMP, isolated from beef heart mitochondria in the presence of MgATP, is relatively low. It increases sharply after a short energization [9–11]. Slow pH-dependent activation of ATPase is observed also in de-energized SMP (Fig. 1). Incubation of S-SMP (depleted of IF_1) under the same conditions does not lead to a significant rise of ATPase activity (data not shown). These data point out that the observed activation is connected with a rearrangement and, perhaps, a dissociation of the E^*IF_1 complex. After complete activation, as well as at a transient stage of activation, ATPase is not inactivated by the fall of pH from 8.0 to 6.7, while further activation is blocked. It is found that the maximal rate of ATP hydrolysis remains constant at varying pH (Fig. 1A) and at a 100-fold change of the SMP concentration in the in-

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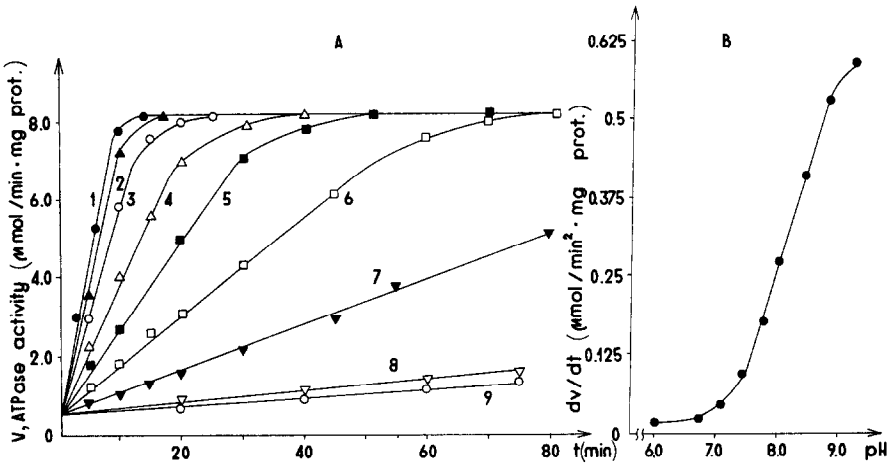

Fig. 1. The pH-dependence of ATPase activation in SMP. (A) SMP (0.88 mg protein/ml) were incubated at 37°C at the following pH values: 9.2 (1); 8.8 (2); 8.4 (3); 8.0 (4); 7.75 (5); 7.45 (6); 7.1 (7); 6.7 (8); 6.0 (9). Components of the incubation medium and conditions of ATPase activity measurements are indicated in section 2. (B) ATPase activation rates were determined by using the initial linear region of the curves from Fig. 1A.

cubation medium (data now shown). These facts are indicative of the irreversible dissociation of IF_1.

The rate of activation sharply drops following a decrease in the ionic strength. At 10 mM K_2SO_4 and pH 8.0 ATPase activity reaches the half-maximal level only after 50 min incubation. Probably, this is the result of a deceleration of E^*IF_1 dissociation. Under these conditions also, no reversion of the activation is observed after the decrease of pH to 6.7.

We used triphenyltin (TPT), which blocks H^+ conductivity through the proton channel of ATPase (F_0) [12], to evaluate a possible role of F_0 in the activation process. Inhibition by TPT (5 μM) was completely reversed by dithiothreitol (4 mM) during the activity measurements. Under the conditions mentioned in the legend to Fig. 1, TPT did not change the kinetics of the activation at any pH investigated.

3.2. *MgATP prevents activation of ATPase*

Addition of MgATP to the incubation medium almost completely prevents ATPase activation. The effect of MgADP was rather weak when hexokinase and glucose were present (Fig. 2). It was established that MgATP is essential for inhibition of ATPase by IF_1 [3], but in our experiments the substrate effect is not caused by the binding of dissociated IF_1. Indeed, a prevention of the activation was observed at the same SMP concentration and pH value as under the conditions of ATPase activity measurements, when no decrease in hydrolysis rate was detected, neither with partially nor with completely activated SMP.

Fig. 3 shows the effect of Mg^{2+} and Ca^{2+} on ATPase activation. The cations significantly slow down the activation. Addition of hexokinase and glucose completely prevents the effect of both cations. Thus their action

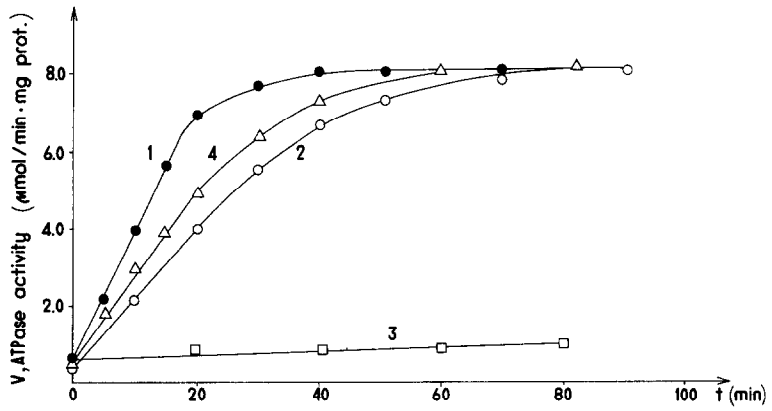

Fig. 2. The effect of adenine nucleotides on kinetics of the ATPase activation. Curve 1, SMP (0.88 mg protein/ml) were incubated at pH 8.0 in the presence of hexokinase (7.8 U/ml), glucose (1 mM) and $MgSO_4$ (0.5 mM). Curve 2, as curve 1, but withoud hexokinase, glucose and $MgSO_4$. Curve 3, incubation medium contained 10 mM phospho*enol*pyruvate, pyruvate kinase (4 U/ml), 3 mM $MgSO_4$, 2 mM ATP. Curve 4, as curve 1 in the presence of 1 mM MgADP. EDTA was omitted from the incubation medium (curves 1–4).

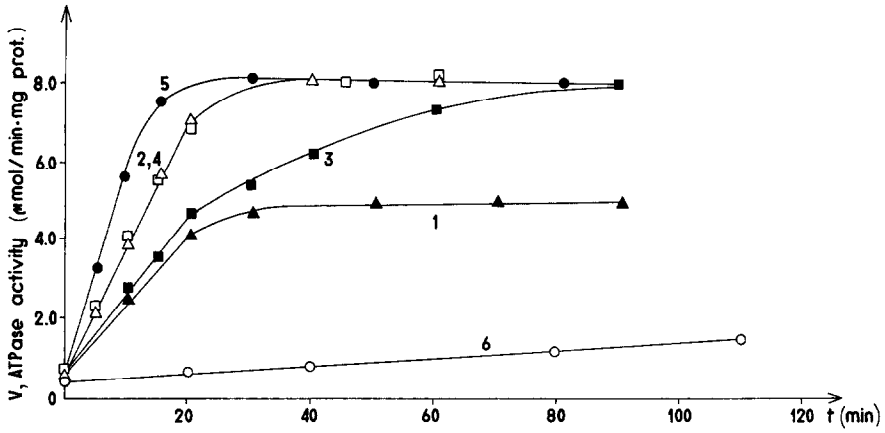

Fig. 3. The effect of Mg^{2+}, Ca^{2+} and Zn^{2+} on ATPase activation. SMP (0.88 mg protein/ml) were incubated at pH 8.0 (curves 1-4) and pH 6.7 (curves 5,6) in the presence (curves 2,4-6) or absence (curves 1,3) of hexokinase (7.8 U/ml), 1 mM glucose and 0.5 mM $MgSO_4$. Additions to the incubation medium were the following: 1 mM $MgSO_4$ (curves 1,2); 1 mM $CaCl_2$ (curves 3,4); 20 μM $ZnSO_4$ (curve 5); no additions (curve 6). EDTA was omitted from the medium (curves 1-6).

is caused by trace amounts of ATP in SMP preparations. The other cation, Zn^{2+} (20 μM) in the presence of hexokinase and glucose accelerates the activation 2-fold at pH 8.0 (data not shown) and more than 10-fold at pH 6.7 (Fig. 3). We have shown earlier that Zn^{2+} anc Cd^{2+} at the same concentrations prevent ATPase inhibition by IF_1 [13]. However, acceleration of the activation is hardly due to the effect of Zn^{2+} on the IF_1 binding. Control experiments showed that in the presence of hexokinase and 0.5 mM Mg^{2+}, the decrease in pH to 6.7 does not inactivate ATPase like in the presence of EDTA (see above). Zn^{2+} does not activate ATPase in the presence of MgATP or at low ionic strength (10 mM K_2SO_4).

4. DISCUSSION

The data presented indicate that activation of ATPase in SMP, isolated in the presence of MgATP, is caused by the dissociation of the E^*IF_1 complex. This process may be described by the following scheme:

$$\begin{array}{l} \qquad\quad H^+ \\ \qquad\quad \diagdown \\ [E^*I^*H]_i \rightleftarrows [E^*I]_i \rightleftarrows [E^*I]_a \rightarrow E + I \\ \qquad\quad \diagdown \\ \qquad\quad H^+ \end{array}$$

where 'i' and 'a' indicate inactive and active enzyme-inhibitor complexes, respectively.

The data in Fig. 1 show that the pH-dependent change of the inactive $[E^*IF_1]$ complex is an essential step in rapid activation. Perhaps, deprotonation of the histidine residue in the IF_1 molecule occurs, resulting in its conformational rearrangement. The existence of this change with $pK = 6.7$ was reported for isolated IF_1 [5,6]. The data presented indicate that the deprotonated form of IF_1 binds to the enzyme significantly weaker than the protonated IF_1, causing an apparent shift of pK of the rearrangement (8.1 in Fig. 1B). It can be proposed that the deprotonated form of IF_1 in the complex with ATPase binds Zn^{2+}, which shifts the equilibrium of the first step of the process and accelerates ATPase activation. This proposal is confirmed by the data that the effect of Zn^{2+} is markedly pronounced at acidic pHs (Fig. 3).

The existence of the catalytically active complex of ATPase with IF_1 directly ensues from the data on MgATP preventing ATPase activation (Fig. 2). An alternative proposal of possible ATP binding and its slow hydrolysis by the initial enzyme-inhibitor complex is not consistent with the data of Kalashnikova et al. [14], who showed that the E^*IF_1 complex does not bind ATP at the active site. Stationary concentration of the active complex is comparatively low, thus ATPase inactivation after acidification of the medium at the transient stages of activation is not observed.

Energization of SMP results in the rapid ATPase activation and in the dissociation of the E^*IF_1 complex [9-11]. Rates of these processes (10 s) are significantly higher than in de-energized SMP even at pH 9.2 (Fig. 1). Perhaps, $\Delta\bar{\mu}H^+$ leads to deprotonation of IF_1 and then accelerates its dissociation. Thus a stationary concentration of the active E^*IF_1 complex drops and MgATP has no effect on its energy-dependent dissociation [11]. The proposed scheme gives an explanation of the discrepancy between the kinetics of energey-dependent activation of ATP hydrolysis (coinciding with the kinetics of IF_1 dissociation) and the more rapid activation of ATP synthesis [9]. Actually, the active E^*IF_1 complex should be rapidly inactivated under the conditions of ATP hydrolysis measurement ($\Delta\bar{\mu}H^+ = 0$, MgATP present), and only the free enzyme

remains active. At initial stages of the activation, this complex may be of principal importance for the ATP synthesis.

The data presented on dissociation of the inhibitor can be adopted for a mechanism of the energy-dependent ATPase activation. Experiments with triphenyltin, inhibitor of ATPase proton channel (F_0), show that the energy-dependent conformational rearrangement in ATPase, leading to a change of *p*K of the essential histidine residue and deprotonation of IF_1 is not dependent on F_0 functioning. Thus the existence of a second proton-translocating pathway, distinct from F_0, crossing part of the membrane and connecting the binding site for IF_1 and the mitochondrial matrix, can be proposed. The apparent *p*K of the group on the bottom of this channel (probably histidine of IF_1) can be shifted for 1–2 units under energization. This proposal accords with the finding that the IF_1 binding suppresses passive H^+ conductivity via the ATPase complex [15]. A tempting idea is that the hypothetical 'second channel' takes part not only in the regulatory deprotonation of IF_1, but also provides (alongside with F_0) proton translocation coupled with hydrolysis and synthesis of ATP.

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