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The interaction of MgADP with H^+-ATPase in rat liver mitochondria

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The activating anions are found to induce an unexpectedly high (up to 8-fold for sulphite) increase of ATPase activity in intact rat liver mitochondria. This effect is not determined by the observed changes in K_m and K_i (ADP) values. The stimulation seems to be caused by dissociation of the inactive complex of ATPase with Mg·ADP. The quantity of this complex formed in the course of ATP hydrolysis is approx. 90% of the total ATPase content in intact mitochondria. The data on toluene-permeabilized mitochondria suggest that the high content of the complex is a result of the stabilizing effect of some matrix macromolecules.

ATPase; Activating anion; Inactive E·MgADP complex; (Rat liver mitochondria)

1. INTRODUCTION

Formation of tight inactive complexes of H^+-ATPase with MgADP plays a central role in the regulation of ATP synthesis and hydrolysis in chloroplasts, some bacteria and, probably, in mitochondria (review [1]). The properties of such complexes with ATPase from beef heart mitochondria were studied by Vinogradov and co-workers (review [2]). It was shown, that at low MgADP concentrations (10^{-8}–10^{-6} M) the inactive complex is formed with $K_d \approx 10^{-8}$ M. This complex dissociates (reactivates) slowly ($k_{-1} \approx 0.2$ min^{-1}) and ATP accelerates the reactivation approx. 10-fold. Sodium azide, the inhibitor of ATPase, prevents dissociation of the complex. In contrast, activating anions (sulphite and bicarbonate) accelerate this process. In our previous work [3] it was shown that formation of inactive complexes with MgADP during ATP hydrolysis at relatively high ADP concentrations (more than 10^{-4} M) is prevented by generation of high $\Delta\mu H^+$ on membrane of submitochondrial particles. These data allow us to propose the $\Delta\mu H^+$-dependent regulation of ATPase with ADP in mitochondria.

In the present work it is established that more than 90% of ATPase molecules are in a tight inactive complex with MgADP during ATP hydrolysis in intact rat liver mitochondria at low $\Delta\mu H^+$. These complexes are probably stabilized by specific proteins of mitochondrial matrix.

Preliminary results of the present work were published [4].

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Abbreviations: SMP, submitochondrial particle; ClCCP, carbonyl cyanide *m*-chlorphenylhydrazone

2. MATERIALS AND METHODS

Rat liver mitochondria and SMPs were isolated according to Pedersen et al. [5]. The treatment of mitochondria with toluene and measurement of glutamate dehydrogenase activity were performed according to Thomas and Denton [6]. SMPs and toluene-treated mitochondria were preincubated for 60 min in a medium containing 250 mM sucrose, 10 mM KCl, 20 mM Tris-HCl, pH 8.0 (SKT), with 1 mM EDTA. ATPase activity of mitochondria (table 1, fig.3) and SMPs (fig.2) was measured by

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P_i production [7] in SKT medium containing 3×10^{-6} M ClCCP and 2 mM MgATP (except fig.2).

The initial rates (fig.2A) were measured in the first 15 s and were found to be unaffected by both sulphite and azide. ATPase activity in the regenerating coupled system [8] was measured spectrophotometrically (fig.1) in SKT medium containing 5 mM $MgSO_4$, 1 mM PEP, 0.3 mM NADH, 2 mM ATP, 5 U/ml pyruvate kinase, 5 U/ml lactate dehydrogenase, 3×10^{-6} M rotenone, 3×10^{-6} M ClCCP.

3. RESULTS AND DISCUSSION

3.1. *Stimulation of ATPase in intact mitochondria by activating anions*

The data presented in table 1 show that the rate of ATP hydrolysis increased approx. 1.5 times in the presence of activating anions. The partial inhibition of ATPase by oligomycin resulted in stimulation of the activity as high as 8-fold (experiments with sulphite). This effect, possibly, reflects the real extent of ATPase stimulation. Low stimulation in control experiments is a result of ATP uptake in mitochondria being a rate-limiting step of ATP hydrolysis in the presence of activating anions. Control experiments on SMPs show that anions do not reactivate ATPase blocked by oligomycin. Partial inhibition of ATP hydrolysis by carboxyatractyloside (specific inhibitor of the ATP/ADP carrier) abolished the effect of the anions (table 1), thus the anions do not change the activity of the carrier.

Table 1

Stimulation of ATP hydrolysis by the anions in intact mitochondria

Conditions of preincubation[a]	ATPase activity (μmol/min per mg protein)		
	No additions	10 mM $KHSO_3$	40 mM $KHCO_3$
No additions	0.220	0.335	0.300
2×10^{-7} M carboxyatractyloside	0.110	0.115	0.110
Oligomycin (0.2 μg/mg protein)	0.103	0.335	n.m.[b]
Oligomycin (0.7 μg/mg protein)	0.029	0.230	0.140

[a] Mitochondria (10 mg protein/ml) were preincubated for 5 min in a medium containing 225 mM sucrose, 20 mM KCl, 10 mM Hepes, pH 7.0, with the additions mentioned in the table

[b] Not measured

3.2. *The effect of activating anions is determined by dissociation of inactive complexes of ATPase with MgADP*

As it was mentioned above the properties of the inactive E·MgADP complex and the effect of sulphite were studied previously on the ATPase from beef heart [2]. Fig.1 shows the properties of the analogous complex in SMPs from rat liver. Preincubation of SMPs with EDTA allows one to measure the initial rate of ATP hydrolysis which slows down to a stationary level in the course of the reaction. The initial rate is not inhibited by azide and is only slightly stimulated by sulphite (fig.1A). Incubation of SMPs with low concentrations of MgADP in intervals (0.1–10 μM) leads to a significant decrease of the initial rate and further a slow reactivation of the ATPase ($\tau_{1/2}$ = 1 min) (fig.1B). The reactivation is immediate in the presence of sulphite and is prevented by addition of azide. Rate of reactivation increases ($\tau_{1/2}$ = 0.2 min) if SMPs were preincubated with high MgADP concentrations (0.1–1 mM). Probably, in these conditions a complex with a different composition is formed (for example, $E \cdot (MgADP)_2$). This complex is also sensitive to azide and sulphite. These data allow one to conclude that all the properties of inactive complexes with MgADP are very similar in rat liver and beef heart ATPases.

The effect of sulphite on competitive inhibition of ATPase by ADP was studied by measuring the initial rates of ATP hydrolysis (fig.2A,B). Sulphite increases K_i (ADP) values from 75 μM to 380 μM and slightly decreases K_m values for ATPase (from 40 μM to 60 μM). These parameters were measured within the MgATP concentration range of 0.1–2 mM.

While ATP hydrolysis in intact mitochondria is blocked by oligomycin (table 1), concentration of free ADP, which is a substrate for the ATP/ADP-carrier, is obviously 2–3-times lower than its K_m value (60 μM) [9]. Knowing the concentration of free Mg^{2+} in the matrix (0.1–1 mM) [10], it is easy to calculate MgADP concentration which would not exceed 0.1 mM. The sum of ADP and ATP concentrations in the matrix is approx. 15 mM [10], so competitive inhibition of ATPase by ADP in our experiments is insignificant. Stimulation of ATP hydrolysis by sulphite in intact mitochondria is determined by dissociation of tight inactive com-

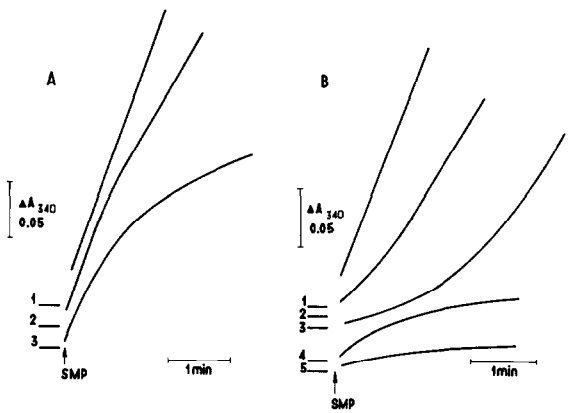

Fig.1. Formation and reactivation of tight inactive complexes of ATPase with MgADP. (A) Curves: 1, 5 mM $KHSO_3$ in the medium; 2, no additions; 3, 0.2 mM NaN_3 in the medium. (B) SMPs were preincubated with 2 mM $MgSO_4$ and 10^{-6} M ADP (curves 1,3,5) or 10^{-4} M ADP (curves 2,4). The additions to the medium were 5 mM $KHSO_3$ (1) and 0.2 mM NaN_3 (4,5). Final SMP concentration was 2.5 μg protein/ml.

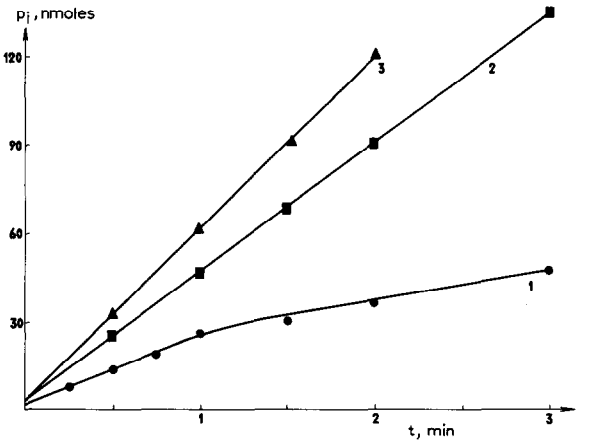

Fig.3. Stimulation by the anions of ATP hydrolysis in toluene-treated mitochondria. In the medium were added 5 mM $NaHSO_3$ (1), 20 mM $NaHCO_3$ (2) or no additions (3). Final mitochondria concentration was 0.175 mg protein/ml.

plexes E·MgADP which number nearly 90% of total amount of ATPases.

3.3. *Matrix factors stabilizing the inactive complex E·MgADP*

Fig.1 shows that stationary rate of ATP hydrolysis by SMPs measured in the presence of pyruvate kinase and PEP is stimulated by sulphite only 2 times (1.5 times by bicarbonate). On the other hand, the effect was 8-fold in intact mitochondria (table 1). This difference may be explained by a higher ADP concentration in mitochondria. Actually the sulphite stimulation of the SMP ATPase stationary rate at relatively low ATP/ADP ratios is high (fig.2C) and is unaffected by 5 mM P_i (not shown). However, at ATP/ADP ratios close to that maintained in the course of ATP hydrolysis in intact mitochondria (see above) the effect of sulphite is not higher than 2–3-fold. Thus another unknown factor stabilizes the inac-

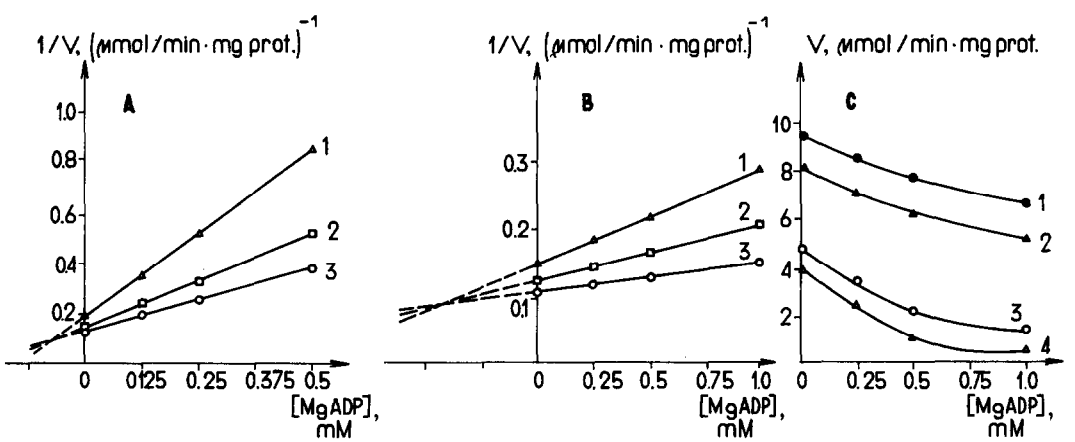

Fig.2. The effect of sulphite on kinetic parameters of ATPase. (A) The initial rates were measured (see section 2). The concentrations of MgATP were 0.125 mM (1), 0.25 mM (2) and 0.5 mM (3). (B) 5 mM $NaHSO_3$ in the medium. The concentrations of MgATP were 0.25 mM (1), 0.5 mM (2) and 1.0 mM (3). (C) 5 mM $NaHSO_3$ in the medium (1,2, ●, ▲), no additions (3,4, ○, △). The concentrations of MgATP were 1.0 mM (1,3) or 0.5 mM (2,4).

tive complex of ATPase with MgADP.

We used mitochondria treated with toluene to study the nature of these factors. ATP hydrolysis after this treatment loses sensitivity to carboxyatractyloside and remains completely sensitive to oligomycin. Measurements of glutamate dehydrogenase activity indicate that this matrix enzyme was not washed away. These data show that in liver mitochondria as in mitochondria from adipose tissue [6], the toluene treatment permeabilizes mitochondria to low molecular mass compounds (nucleotides) but not to macromolecules.

High initial rate of ATP hydrolysis in these mitochondria decreases in 2.5 times reaching a stationary level that increases 5-fold in the presence of sulphite and 3-fold in the presence of bicarbonate (fig.3). Consequently, hypothetical matrix components, which stabilize the inactive complex E·MgADP, are the compounds of high molecular mass, probably proteins. The rate of ATP hydrolysis in toluene-treated mitochondria in the presence of pyruvate kinase and PEP (when the ADP concentration does not exceed 10 μM) is stimulated by sulphite no more than 2-fold (not shown). Thus we propose that in intact mitochondria only inactive complex, formed at high ADP concentrations, is stabilized by specific matrix factors. Their nature is now under investigation.

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