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INTERACTION OF Mg^{+2} WITH BEEF HEART MITOCHONDRIAL ATPase (F_1)

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Summary. The soluble mitochondrial ATPase, F_1, can be slowly inactivated by incubation with Mg^{+2} in a manner consistent with the observations of Moyle and Mitchell (FEBS Lett. 56, 55 (1975)). This inhibition results in a low initial rate of ATP hydrolysis upon addition to an ATPase assay medium of F_1 which has been incubated with Mg^{+2}. This inhibition, however, is completely reversible by Mg·ATP in a time dependent process and results in the rate of ATP hydrolysis increasing during the ATPase assay to reach control levels after 30 sec. The length of the lag is independent of the F_1 concentration in the ATPase assay and the lag is also completely reversed by subsequent incubation with excess EDTA before assay.

F_1 is unstable if incubated with EDTA in the absence of free nucleotides or Mg^{+2}. The rate of inactivation increases with decreasing protein concentration until a limiting rate is reached at high dilution. Mg^{+2} in excess of the EDTA or 50 μM ADP stabilize the F_1 against the inactivation but cannot reverse prior denaturation.

The kinetics of ATP hydrolysis by the mitochondrial ATPase are complex and slow responses of F_1 to changes in a number of experimental conditions have been noted (1-7). Moyle and Mitchell (2) have reported that beef heart F_1 is inactivated by incubation with Mg^{+2} in a slow process and inactivation of the rat liver enzyme by Mg^{+2} has also been reported (8). The experiments reported here confirm this observation and further show that Mg·ATP completely reverses the inhibition in 30 seconds and that a direct interaction of Mg^{+2} with the enzyme can also be demonstrated by the ability of Mg^{+2} to protect F_1 against irreversible inactivation at high dilution.

METHODS

F_1 ATPase. Beef heart mitochondria were prepared essentially according to the method of Smith (9) and submitochondrial particles were prepared by

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sonification in 250 mM sucrose, 10 mM Tris·Cl pH 8.0 and 1 mM EDTA. F_1 was released from the membrane by $CHCl_3$ treatment (10) and purified by ion exchange chromatography (11).

The F_1 was washed by two cycles of suspension of the $(NH_4)_2SO_4$ pellet in 60% saturated $(NH_4)_2SO_4$ and centrifugation. The final pellet was dissolved in a minimal amount of 150 mM KCl, 50 mM Tris·Cl pH 8.0 and 1 mM EDTA and rapidly chromatographed on a 0.7 x 12 cm column of Sephadex G50. The peak fraction was frozen in dry ice/acetone in small aliquots and stored at -70° until immediately before use. Protein concentration was determined by the method of Lowry (12) using serum albumin as standard (13).

Kinetics. The ATPase reaction was followed spectrophotometrically using an ATP regenerating system. The assay mixture contained 50 mM Tris·Cl pH 8.0, 20 mM KCl, 1 mM EDTA, 5 mM $MgCl_2$, 5 mM Mg·ATP, 1 mM phosphoenolpyruvate, 0.25 mM NADH, 80 U pyruvate kinase and 90 U lactate dehydrogenase at 25°. The F_1 was incubated as indicated and a small aliquot was applied to a cuvet stirrer. The reaction was initiated by three rapid plunges of the cuvet stirrer to the bottom of a cuvet containing the complete reaction mixture minus F_1. Pyruvate kinase and lactate dehydrogenase were obtained from Sigma Chemical Company as salt-free lyophylized powders.

RESULTS AND DISCUSSION

No lag is observed on the time scale indicated in Figure 1 in the onset of ATP hydrolysis by F_1 which has been incubated in EDTA buffer although a

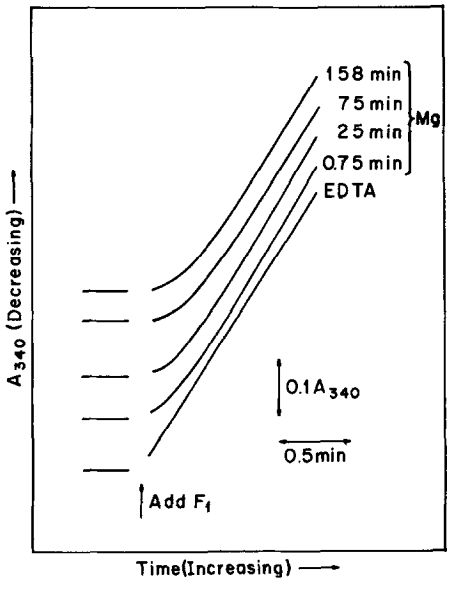

Figure 1. Time course during spectrophotometric ATPase assay. The F_1 stock solution at 8.4 mg/ml was diluted to 168 µg/ml in 50 mM Tris·Cl pH 8.0, 20 mM KCl, 1 mM EDTA and after 1 min., 168 µg were removed for the ATPase assay designated EDTA. ATPase assays with F_1 which was incubated similarly for increasing times in the above buffer supplemented with 5 mM $MgCl_2$ are designated Mg.

short lag in the milisecond time range is likely to be occurring (14). The addition of Mg^{+2} in excess of the EDTA to the incubation medium of the F_1, however, produces a pronounced lag in the onset of ATP hydrolysis and approximately 30 seconds are required before the steady rate is reached. Although a significant lag is present at 0.75 minutes after addition of Mg^{+2}, maximum development of the lag requires longer incubation. The increase in hydrolysis rate during the assay seen in Figure 1 indicates that the inhibition is completely reversible by Mg·ATP. The recovery process during ATP hydrolysis is not likely to be due to dissociation/reassociation reactions of the subunits of F_1 as no change in the recovery rate is observed over a 10 fold range of F_1 concentrations in the assay (not shown).

The inhibition by free Mg^{+2} can be completely reversed by subsequent addition of EDTA in excess of the Mg^{+2} in the incubation medium and again several minutes are required before the excess EDTA can reverse the effect of the incubation in the presence of free Mg^{+2} (not shown). This observation implies that the Mg^{+2} - induced inactivation is due to Mg^{+2} binding itself and not merely due to a Mg^{+2} - catalyzed hydrolysis of any residual free ATP or tightly bound ATP, although it cannot be excluded that such hydrolysis is necessary for the inactivation. The direct binding of EDTA to F_1 is also possible (15,16).

Further evidence for a direct interaction of Mg^{+2} with F_1 comes from the ability of Mg^{+2} to stabilize the enzyme against irreversible inactivation at high dilution. When F_1 is diluted in EDTA buffer, it becomes unstable and the rate of inactivation increases until a plateau rate is reached below 5 μg F_1/ml. Figure 2 shows the decrease in ATPase rate observed with incubation of F_1 at low concentration in buffer containing EDTA. The rate of loss of ATPase activity is biphasic representing either heterogeneity in the F_1 or a uniform transition to a partially active state. The activity which is resistant to inactivation still exhibits a lag on subsequent incubation with free Mg^{+2}. When the incubator buffer is supplemented with 5 mM $MgCl_2$

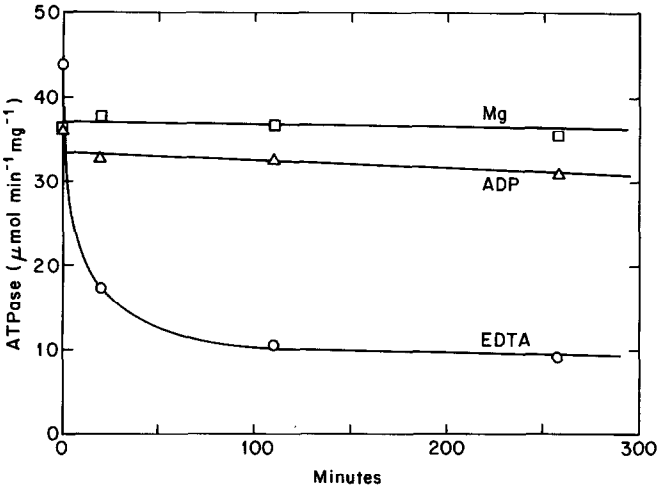

Figure 2. Instability of F_1 on dilution in EDTA buffer and stabilization by Mg^{+2} or ADP. The F_1 stock solution at 8.4 mg/ml was diluted to 10.5 μg/ml in 50 mM Tris·Cl pH 8.0, 20 mM KCl, 1 mM EDTA alone (O) or supplemented with 5 mM $MgCl_2$ (□) or 50 μM ADP (Δ). The steady state rate of ATP hydrolysis was determined on aliquots at increasing times as indicated.

or 50 μM ADP the inactivation by dilution is prevented although the rate after incubation with ADP is lower than the initial value as observed by Harris et al. (6). The ATPase rate reported for enzyme incubated with EDTA represents the initial rate while the ATPase rates reported following incubation with Mg^{+2} or ADP represent steady state rates obtained after recovery from the Mg^{+2} - induced lag and after any burst caused by addition of ADP to the assay mixture. Direct Mg^{+2} binding to F_1 is consistent with the stimulation by Mg^{+2} of tight P_i binding observed by Penefsky (16).

The inactivation induced by dilution in EDTA buffer is irreversible unlike the effect of incubation with Mg^{+2}. No increase in ATPase rate with the dilution-inactivated F_1 is observed during the ATPase assay and furthermore, although Mg^{+2}, ADP or ATP can prevent dilution-induced inactivation, they are unable to reverse inactivation which has already occurred as shown in Figure 3.

It should be pointed out that although the experiments reported here were performed using F_1 which had been released by $CHCl_3$ and had undergone gel filtration at moderate ionic strength and may be partially depleted in

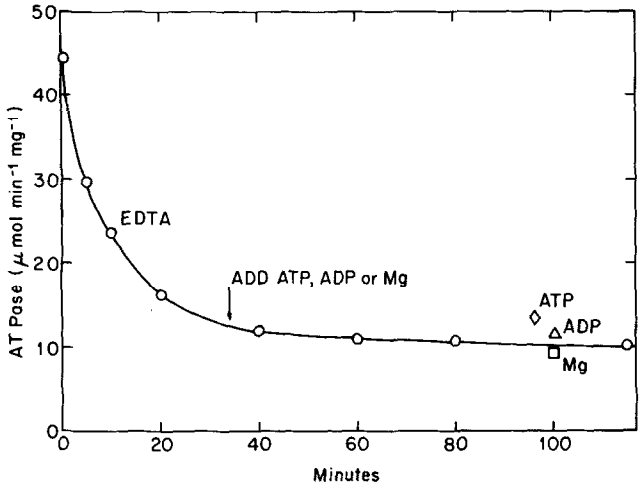

Figure 3. Inability of Mg^{+2}, ADP or ATP to reactivate F_1 inactivated by dilution in EDTA buffer. F_1 was incubated in EDTA buffer as in Figure 2 (O) and at time indicated by the arrow portions were made 5 mM in $MgCl_2$ (□), 100 μM in ADP (△) or 1.0 mM in ATP (◇).

tightly bound nucleotides (17), essentially identical results have been obtained using F_1 prepared by the procedure of Knowles and Penefsky (18) and subsequently chromatographed on Sephadex G50 at low ionic strength.

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