Kinetic Properties of Mg, Ca ATPase from Various *Escherichia coli* Mutants

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The ATPase is not lacking in the ATPase mutants DL 54 and AN 120 but has very different kinetic properties including a higher Cl^- optimum and higher K_m values for MgATP. In AN 120, the ATPase activity also has a higher Mg^{2+} optimum.

Introduction

ATPase mutants and uncoupled mutants have played an important part in establishing the function of the Mg, Ca ATPase in oxidative phosphorylation for review see ref. 1). Since the uncoupled mutant etc-15 has a high ATPase activity (about half of [2] or the same as the wild type [3]), it was suggested that this mutated ATPase is less tightly bound to the membrane [2]. However, the mutation in etc-15 seems to be in the γ-subunit [2], whereas the δ-subunit, which is unchanged in etc-15, is responsible for membrane binding [4]. The ATPase mutant AN 120 has considerable ATPase activity, when tested at high salt concentration [5]. Thus, it is unclear whether the reduced ATPase activity of these mutants is a consequence of lacking or of reduced binding of ATPase to the membrane, of inactive ATPase, or of an ATPase with altered kinetic properties. Therefore, we tested the kinetic properties of the ATPase from the wild type *E. coli* ML 308-225, from its mutant etc-15 with uncoupled electron transfer, and from the ATPase deficient mutants DL 54 and AN 120.

Materials and Methods

E. coli ML 308-225, etc-15 and DL 54 were grown in a mineral glucose medium. The ATPase mutant *E. coli* K 12, AN 120 was grown in the same medium with addition of 0.2 μM thiamine and 0.2 mM arginine [5] and *E. coli* B 163 with addition of leucine, histidine and methionine [6]. The preparation of membrane-bound (lysozyme-method) and of solubilized ATPase and the determination of enzyme activity have been extensively described [7, 8]. The K_m and V-values were determined according to Lineweaver and Burk [9] and Eisenthal and Cornish-Bowden [10].

The wild type ML 308-225, the mutants etc-15 and DL 54 were generous gifts from P. G. Bragg and R. D. Simoni and were used directly in the experiments. AN 120 was an earlier gift from F. Gibson, and since periodically selected by U. Zimmermann, Jülich.

Results and Discussion

The wild type *E. coli* ML 308-225 and the derived mutants etc-15 and DL 54 have the same pMg optima (Table I). At low Mg^{2+} concentrations the ATPase is activated and at high Mg^{2+} concentrations it is inhibited. The Mg^{2+} optimum was independent of the Cl^- concentration (not shown). At high MgATP concentration, the Mg^{2+} optimum is shifted to higher Mg^{2+} concentration. This behaviour has been described in detail for *E. coli* B 163 and analysed as being a competitive inhibition of MgATP hydrolysis by Mg^{2+} [7]. Hence, this property is not mutated in these strains. However, the other ATPase mutant tested, AN 120, shows a Mg^{2+} optimum at higher Mg^{2+} concentrations than the other strains (Table I).

The ATPase of all strains tested was activated by KCl (Table I). The effect of other salts was previously described and analysed as being a specific effect of anions. Effects of osmotic pressure and ionic strength could be excluded [6]. The Cl^- optimum for the wild type ML 308-225 was in the same range as in another wild type *E. coli* B 163 [6], and independent of the concentration of MgATP. The uncoupled mutant etc-15, in which the ATPase activity at low MgATP concentrations is 50% of the wild type, has the same Cl^- optimum. However, in the mutants DL 54 and AN 120, in which there is even less ATPase activity, the remaining ATPase of each strain has a Cl^- optimum at 90 mM Cl^-. The Hill-coefficient for the Cl^- activation in all strains is greater than 2 (not shown). The K_m values of wild type ATPase increases with decreasing Cl^- concentration in the range from 0.01 – 0.07 mM [6], whereas the K_m values of the mutants etc-15, DL 54 and AN 120 are not significantly dependent on the Cl^- concentration. They amounted to 0.07 mM for etc-15 and AN 120 and 0.12 mM for DL 54. Thus the activation of these strains by Cl^- comes from an increase in their V (Table I). These strains reach considerable ATPase activity, when optimal conditions are chosen for each. Thus there is no lack of ATPase in the ATPase mutants DL 54 and AN 120, but the mutant ATPase has extremely changed properties, namely a decreased affinity for

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Table I. pMg optimum, Cl^- optimum (mM) and ATPase activities of various *E. coli* strains. ATPase activities v, V are given in nmol Pi/min·mg protein. Membrane-bound ATPase was used. Incubation was performed in 10 mM Tris Cl buffer, pH 8.5, at 37 °C. pMg = $-\log[Mg^{2+}]$, Mg^{2+} ion concentration was calculated from the Mg ATP-stability constant as already described [7, 8].

pMg	—	—	4.4	4.4	4.4	4.4	4.4	4.0	4.0	4.0	4.0	4.0
$[Cl^-]$ mM	30	30	—	7	30	90	200	3	10	30	50	90
[Mg ATP] M	1.5×10^{-5}	3.5×10^{-4}	8×10^{-6}	8×10^{-6}	8×10^{-6}	8×10^{-6}	8×10^{-6}	—	—	—	—	—
E. coli strain	pMg-Optimum		Cl^--Opt.	v				V				
ML 308-225	4.7	3.9	30	13.6	17.0	10.0	—	26	28	30	—	—
etc-15	4.7	3.9	30	3.0	7.8	3.8	—	13	20	28	—	—
DL 54	4.7	3.9	90	0.7	0.9	3.9	1.6	—	—	11	17	21
AN 120	4.4	3.5	90	0.1	0.3	3.5	1.3	—	—	6	20	25

substrate (Mg ATP), Cl^-, and in the case of AN 120, additionally for Mg^{2+}. At low substrate and salt concentrations we also obtained the low activities of the mutants, reported in the literature [2, 3, 5, 11]. Therefore we suggest that under the conditions within the intact cell the ATPase activity of the mutants may be not sufficient to perform ATPase-dependent membrane functions.

As azide is a strong inhibitor of *E. coli* ATPase [12, 13], and as its action is counteracted by KCl [13], we tested its effect on the ATPase activity of the various *E. coli* strains.

With 0.5 mM ATP and 0.5 mM $MgCl_2$ in 25 mM Tris Cl buffer, pH 8.5 membrane-bound and solubilized ATPase from B 163, ML 308-225 and etc-15 was strongly inhibited. We have found half maximal inhibition at about 10^{-4} M azide. However, the ATPase from DL 54 and AN 120 was not inhibited under these conditions, up to 3 mM azide. These results show that the azide-sensitive site is mutated in DL 54 and AN 120.

Since DL 54 and AN 120 have a decreased affinity for anions, it is possible that the azide-binding site may influence the activation process by anions. For characterization of this site, it may be interesting that Zn^{2+} was found to be essential for *E. coli* ATPase [14] and that Zn^{2+} is able to react with azide [15].

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