The Membrane ATPase of the (Mg, Ca)-ATPase-Mutant *E. coli* K 12, strain AN 120

Theodor Günther and Gert Mariß

Zentralinstitut für Biochemie und Biophysik, Freie Universität Berlin

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(Mg, Ca)-ATPase-Mutant

The activity of the membrane ATPase of the (Mg, Ca)-ATPase-mutant *E. coli* K 12 strain AN 120 is reduced to about 5–50% of the wildtype ATPase. The reduction is greatest at pH 9.1 and a ratio of Mg, or Ca to ATP of 1:5, and smallest at pH 7.5 and 9.1 and a ratio of Mg or Ca to ATP of 1 or more.

In the (Mg,Ca)-ATPase-mutant *E. coli* K 12 strain AN 120, isolated by Butlin *et al.*[1], the activity of the membrane ATPase and of the ATP-dependent transhydrogenase[2] is greatly reduced. These cells are not able to grow on succinate or D-lactate because they lack oxidative phosphorylation. In the course of testing the properties of membrane ATPase, we found, in addition to the reduction of activity, qualitative differences in the remaining ATPase activity with respect to the activation by Mg or Ca and the effect of salts.

Methods

E. coli K 12, AN 120 was cultured in the glucose-mineral medium, described earlier[3] with the addition of 0.2 mM arginine and 0.2 μM thiamine. Bacteria from freshly selected colonies were used. The preparation and testing of the ATPase were described in ref.[4].

Results

The remaining Mg-ATPase of the (Mg, Ca)-ATPase-mutant has a pH dependence similar to that of the wildtype ATPase (Fig. 1, for comparison see[4]). The effect of alkali chlorides at pH 7.5 and 9.1 and a Mg:ATP ratio of 5 : 5 is the same as that for the wildtype ATPase (Fig. 1 and Table I). A greater difference was found at pH 9.1 and a Mg: ATP ration of 1:5. Under these conditions the increase in the mutant ATPase activity is as much as 300%, while in the wildtype the high activity is inhibited[4].

Requests for reprints should be sent to Professor Dr. T. Günther, Zentralinstitut für Biochemie und Biophysik, *D-1000 Berlin 33*, Arnimallee 22.

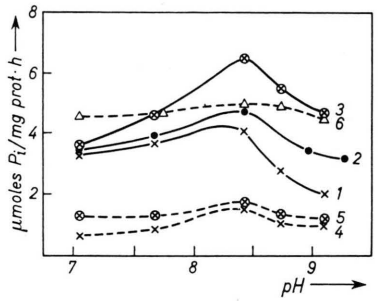

Fig. 1. pH-Dependence of the membrane ATPase of the (Mg, Ca)-ATPase-mutant *E. coli* K 12 strain AN 120. The Mg/ATP- (solid lines) and Ca/ATP-ratio (broken lines) always was 5/5. Curve 1, MgATP; 2, MgATP; 3, MgATP + 0.3 M KCl; 4, CaATP; 5, CaATP + 0.3 M KCl; 6, CaATP + 0.3 M Na acetate. Curve 2: Tris Cl-buffer with constant Cl^--concentration. Other curves: Tris Cl-buffer with constant Tris-concentration.

Table I. Effect of various salts on Mg-ATPase and Ca-ATPase of AN 120 at pH 7.5 and pH 9.1 (0.1 M Tris Cl-buffer, constant Tris-concentration) with 1 or 5 mM Mg or Ca respectively. The ATP concentration was always 5 mM. Salt-concentration 0.3 M (μmoles P_i/mg prot·h).

	Mg			Ca		
pH	7.5	9.1		7.5	9.1	
Me/ATP (mM/mM)	5/5	1/5	5/5	5/5	1/5	5/5
ϕ	3.6	1.3	2.1	0.6	1.4	1.0
Na acetate	—	—	—	4.0	4.2	4.4
$NaHCO_3$	3.0	0.8	1.3	0.7	0.8	0.9
LiCl	2.7	2.0	3.0	1.2	1.1	1.0
NaCl	2.9	2.0	3.9	1.0	1.2	1.0
KCl	3.6	3.5	4.5	1.1	1.3	1.2
RbCl	3.2	4.0	4.0	1.2	1.4	1.2
CsCl	2.4	4.0	3.5	1.1	1.2	0.9
NaJ	1.7	1.3	2.0	1.0	1.0	1.2
$NaNO_3$	1.6	1.4	1.8	1.0	0.8	0.9
NaSCN	0.9	0.6	0.8	0.6	0.8	0.8

Abbreviations: ATPase, (Mg, Ca)-ATPase, Mg- and Ca-dependent adenosine triphosphatase [E.C. 3.6.1.3]; Mg-ATPase, Ca-ATPase, activity of the (Mg, Ca)-ATPase in presence of Mg or Ca; Mg/ATP = 5/5, (Mg) = 5 mM and (ATP) = 5 mM.

The Ca-ATPase from AN 120 is strongly activated by acetate (Table I and Figs 1, 3). As in the wildtype, the acetate-dependent ATPase activity is largely independent of pH. Although the Ca-ATPase of the wildtype is strongly inhibited by most of the salts tested, they have little effect on the mutant enzyme (Table I). There was thus only a slightly apparent increased inhibition of the mutant ATPase along a chaotropic series of anions. The most striking difference is the dependence of ATPase activity on Mg- or Ca-concentration (Figs 2, 3). At pH 9.1 the peak at 1 mM Mg or Ca (in presence of

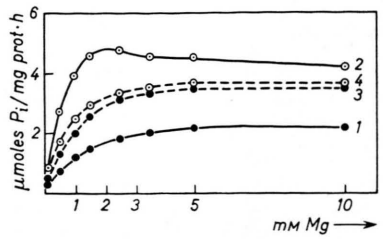

Fig. 2. Effect of Mg-concentration on ATPase activity of *E. coli* K 12, strain AN 120. Curve 1, pH 9.1; 2, pH 9.1 + 0.3 M KCl; 3, pH 7.5; 4, pH 7.5 + 0.3 M KCl.

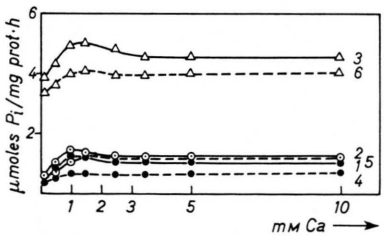

Fig. 3. Effect of Ca-concentration on ATPase activity of *E. coli* K 12, strain AN 120. Curve 1, pH 9.1; 2, pH 9.1 + 0.3 M KCl; 3, pH 9.1 + 0.3 M Na acetate; 4, pH 7.5; 5, pH 7.5 + 0.3 M KCl; 6, pH 7.5 + 0.3 M Na acetate.

5 mM ATP) is completely missing in the ATPase mutant. Under these conditions the ATPase-activity we observed is in agreement with that reported by Butlin *et al.* [1], being only 5% of the wildtype. At 5 mM Mg or Ca, however, the mutant ATPase amounts to about 50% of the wildtype. Hong and Kaback [5] also found half the wildtype activity under comparable conditions. The effects of inhibitors on the ATPase of AN 120 correspond, in so far as tested, to the effects of inhibitors on the wildtype.

Discussion

The ATPase activities of membrane vesicles from different strains of *E. coli* are identical, if tested under the same conditions [5]. *E. coli* K 12 [6]- and *E. coli* B [4]-ATPase activities show the same Mg- and Ca-dependence. The decrease in ATPase is the same when compared to the parent strain AN 180 [1] or *E. coli* B 163 [4] as reference. Thus *E. coli* B 163 can serve as a control. The most striking difference between widltype- and mutant-ATPase is the missing peak of activity at 1 mM Mg or Ca (with 5 mM ATP at pH 9.1). The Mg- and Ca-activity peak of the wildtype ATPase at pH 9.1 may be due to additional sites on the enzyme which are activated at Mg- or Ca-concentrations below 1 mM and inhibited between 1 and 5 mM, the activation being prevented by the presence of salt. This particular part of the enzyme may be mutated in AN 120. Another possibility is that the (Mg, Ca)-ATPase activity of the peak is a separate enzyme, which may be inactivated by the mutation.

The uncoupling of oxidative phosphorylation may be due to a lack of ATPase, in particular of the ATPase active a 1 mM Mg. (Mg is the physiological activator.) However, the ATPase activity of AN 120 in the presence of a rather high Mg-concentration, as is the case in the intact bacterium [7], still amounts to 50% of the wildtype activity. Thus it is possible that uncoupling of oxidative phosphorylation in AN 120 is produced by the accompanying lack of ATP-dependent transhydrogenase. This conclusion is confirmed by the isolation of *E. coli* mutants defective in oxidative phosphorylation and having a lack of ATP-driven transhydrogenase but unchanged (Mg, Ca)-ATPase activity [8, 9]. As the lipid composition has some effect on the action of salts [10], it is possible that the changed lipid composition of AN 120 has a modifying action on the ATPase activity. There is an increased content of phosphatidylglycerol (from 8.5% to 19.2%) and a decreased content of cardiolipin (from 11.3% to 4.7%) the other fractions being unchanged (unpublished). A similar correlation has been found in mitochondria. A reduction of the cardiolipin concentration was accompanied by the same decrease in ATPase activity [11].

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