Activation and Inhibition of the Mg-Ca-ATPase from *E. coli* by Mg²⁺ and Ca²⁺

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(Z. Naturforsch. 30 c, 412—416 [1975]; received January 7, 1975)

**Mg-Ca-ATPase, E. coli, Activation, Inhibition**

MgATP or CaATP is the substrate of the Mg-Ca-ATPase. At low Mg²⁺ or Ca²⁺-concentrations the ATPase is activated by Mg²⁺ or Ca²⁺, the activator being essential for activity. At higher Mg²⁺ or Ca²⁺-concentrations the Mg-Ca-ATPase is inhibited competitively. Thus the real *Kₘ* is smaller than reported in the literature. H⁺ competes with Mg²⁺ or Ca²⁺ for the metal binding sites.

**Introduction**

The Mg-Ca-ATPase of *E. coli* is involved in oxidative phosphorylation and in ATP-dependent membrane functions (active transport of amino acids and K⁺ under anaerobic conditions, ATP-driven transhydrogenase). The enzymatic properties of the Mg-Ca-ATPase were extensively studied (for literature see ref. 4).

However, the effect of the single components of the substrate (Mg²⁺, Ca²⁺, ATP, MgATP, CaATP) on *E. coli* ATPase activity is not defined. For analysing these complex relations we examined the MgATP-dependence at constant pH values and the pH dependence at constant MgATP-concentrations.

**Materials and Methods**

**Symbols and Abbreviations:** S = substrate (MgATP, CaATP); P = product(s); [Mg²⁺] = concentration of free metal ions [Mg²⁺, Ca²⁺]; [Mg²⁺]ₜ, [ATP]ₜ = total concentrations; pMe = negative logarithm of metal ion concentration.

**Preparation of the Mg-Ca-dependent ATPase**

*E. coli* strain B 163 was cultured as described by Günther and Dorn. The ATPase was prepared as described by Evans.

**Determination of enzyme activity**

The enzyme activity was tested at 37 °C in 10.0 ml 100 mm Tris buffer, titrated with 2 N HCl to pH 7.5, 8.2 and 9.1. After 10 min preincubation with MgCl₂ or CaCl₂ the reaction was started by adding ATP. Total ATP-concentrations amounted to 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.40, 0.50, 1.00 and 1.50 mM. To each [ATP], the following [Mg²⁺] or [Ca²⁺] were added: 0.02, 0.05, 0.10, 0.20, 0.30, 0.50, 1.00, 2.00 mM.

The reaction was followed by continuously measuring the release of inorganic phosphate, as described by Ahlers. In order to dissolve the membranes, 6% sodium dodecylsulfate was added continuously together with TCA. Care was taken to ensure that the hydrolysis of the substrate amounted to 5% but never exceeded 10% of the total substrate concentration. So the measurements were always being made in the area of linearity and under steady-state conditions.

Protein was determined by the method of Lowry et al. using bovine serum albumin as a standard.

**Calculation of [MgATP], [CaATP], [ATP], [Ca²⁺] and [Mg²⁺]**

From the 80 mixtures of Me²⁺ and ATP at each pH the resulting concentrations of MeATP were calculated using the following equation:

\[
[M_{\text{MeATP}}] = \frac{[M_{\text{Me}^2+}] + [\text{ATP}] + K_{\text{MeATP}}}{2} - \sqrt{\left(\frac{[M_{\text{Me}^2+}] + [\text{ATP}] + K_{\text{MeATP}}}{2}\right)^2 - [M_{\text{Me}^2+}] [\text{ATP}]}\]

with \(K_{\text{MeATP}} = 2.15 \times 10^{-4} \text{ M} \) (ref. 11) and \(K_{\text{CaATP}} = 7.25 \times 10^{-4} \text{ M} \) (ref. 12, cited from 13).

From the obtained [MeATP] the concentrations of free ATP and free Me²⁺ were calculated using

\[
[M_{\text{Me}^2+}] = [M_{\text{Me}^2+}] - [\text{MeATP}]\]

and

\[
[\text{ATP}] = [\text{ATP}] - [\text{MeATP}].
\]

From these values the reaction rates for constant [MeATP] at varying [Me²⁺] (as shown in Figs 1–3, 6, 8) and for constant [Me²⁺] at varying [MeATP] (Figs 5, 7) were interpolated.
Derivation of the rate equation for the reaction

The derivation of the rate equation was performed on the basis of a rapid equilibrium reaction according to models presented by Botts and Morales 14, Laidler 15, Ohlenbusch 16, and Cleland 17.

Chemicals

ATP was obtained from Boehringer, Mannheim, Germany. All other chemicals were purchased from E. Merck, Darmstadt, Germany, and were of reagent grade.

Results

The reaction rates at various but constant concentrations of MgATP and H+ ions are plotted as a function of pMg (Figs 1—3). These figures show that the Mg2+-ions activate the E. coli ATPase at low concentrations and inhibit the enzyme at higher concentrations. The optima of the obtained v — pMg functions are shifted to higher metal concentrations with increasing substrate concentration and with increasing hydrogen ion concentration.

From the values in the ascending parts of the curves in Fig. 1 \( \frac{1}{v} = f(1/[Mg^{2+}]) \) at constant [MgATP] and \( \frac{1}{v} = f(1/[MgATP]) \) at constant [Mg2+] have been plotted (Figs 4 and 5). Within experimental error the obtained straight lines have
a common point of intersection on the abscissa or in the third quadrant. From the values of the descending parts of the curves in Figs 1—3 \( \frac{1}{v} \) was plotted against \([\text{Mg}^{2+}]\) at constant \([\text{MgATP}]\) (e.g. Fig. 6) and \( \frac{1}{v} \) against \( \frac{1}{[\text{MgATP}]} \) at constant \([\text{Mg}^{2+}]\) (e.g. Fig. 7). We obtained straight lines with a common point of intersection on the ordinate (\( \frac{1}{v} \) vs \([S]\)) or in the second quadrant (\( \frac{1}{v} \) vs \([\text{Mg}^{2+}]\)). With \( \text{Ca}^{2+} \) instead of \( \text{Mg}^{2+} \) corresponding results were observed (not shown).

However, the plot \( \frac{1}{v} \) vs \([\text{Mg}^{2+}]\) at pH 9.1 was not linear, but \( \frac{1}{v} \) vs \([\text{Mg}^{2+}]^2\) was linear (Fig. 8). From the slopes, the ordinate values and from the common points of intersections of the straight lines (e.g. Figs 5—8), the kinetic constants \( K_m, V, K_A \) and \( K_I \) were calculated (Table I).

**Discussion**

Since the solubilized and purified enzyme is too unstable for reproducible kinetic measurements and for testing the ATPase-complex in a more physiological state we used the membrane bound enzyme.

**Table I. Kinetic constants for Mg, Ca-ATPase from *E. coli* B163.** Values were taken from \( \frac{1}{v} \) vs \([S]\), \( \frac{1}{v} \) vs \([\text{Mg}^{2+}]\), \( \frac{1}{v} \) vs \([\text{Mg}^{2+}]^2\) (e.g. Figs 4—8) and from the secondary plots (slopes and ordinate values of Lineweaver-Burk- and Dixon-plots vs effector concentration or \( \frac{1}{[S]} \) resp.). \( K_m \) and \( V \) are real constants (\( [S] \rightarrow \infty, [A] \rightarrow \infty \) resp. \([I] \rightarrow 0\)).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>pH</th>
<th>Effector</th>
<th>Acting as</th>
<th>( \frac{V}{[\text{mmol P_i}]} )</th>
<th>( K_m )</th>
<th>( K_A )</th>
<th>( K_I )</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgATP</td>
<td>7.5</td>
<td>( \text{Mg}^{2+} )</td>
<td>activator</td>
<td>0.23 ( \text{μmol P_i [min} \cdot \text{mg prot]} )</td>
<td>20 ( \text{μM} )</td>
<td>15 ( \text{μM} )</td>
<td>70 ( \text{μM} )</td>
</tr>
<tr>
<td>MgATP</td>
<td>7.5</td>
<td>( \text{Mg}^{2+} )</td>
<td>inhibitor</td>
<td>0.21 ( \text{μmol P_i [min} \cdot \text{mg prot]} )</td>
<td>10 ( \text{μM} )</td>
<td>12 ( \text{μM} )</td>
<td>40 ( \text{μM} )</td>
</tr>
<tr>
<td>MgATP</td>
<td>8.2</td>
<td>( \text{Mg}^{2+} )</td>
<td>inhibitor</td>
<td>0.36 ( \text{μmol P_i [min} \cdot \text{mg prot]} )</td>
<td>30 ( \text{μM} )</td>
<td>30 ( \text{μM} )</td>
<td>35 ( \text{μM} )</td>
</tr>
<tr>
<td>MgATP</td>
<td>9.1</td>
<td>( \text{Mg}^{2+} )</td>
<td>inhibitor</td>
<td>0.56 ( \text{μmol P_i [min} \cdot \text{mg prot]} )</td>
<td>47 ( \text{μM} )</td>
<td>9 ( \text{μM} )</td>
<td>64 ( \text{μM} )</td>
</tr>
<tr>
<td>CaATP</td>
<td>7.5</td>
<td>( \text{Ca}^{2+} )</td>
<td>activator</td>
<td>0.16 ( \text{μmol P_i [min} \cdot \text{mg prot]} )</td>
<td>90 ( \text{μM} )</td>
<td>9 ( \text{μM} )</td>
<td>64 ( \text{μM} )</td>
</tr>
<tr>
<td>CaATP</td>
<td>9.1</td>
<td>( \text{Ca}^{2+} )</td>
<td>inhibitor</td>
<td>0.50 ( \text{μmol P_i [min} \cdot \text{mg prot]} )</td>
<td>90 ( \text{μM} )</td>
<td>9 ( \text{μM} )</td>
<td>64 ( \text{μM} )</td>
</tr>
</tbody>
</table>
1/[A] show that one molecule of substrate and one activator ion react per active centre. If there are more ATP-splitting centres within the ATPase complex as probable, they are not interacting. The activator ions are essential for activity. The following model for activation is in agreement with the results:

\[
\begin{align*}
ES & \rightleftharpoons k'_1 E + k_a A \\
& \rightleftharpoons k_1 E A + k_2 E A S \rightarrow E A + P
\end{align*}
\]

The substrate molecule can be bound by the free enzyme E and by the enzyme-activator-complex EA. However, only the enzyme-activator-substrate-complex EAS decomposes. This model results in the following reaction rate equation under equilibrium conditions:

\[
v = \frac{V \cdot [S]}{K_m \cdot [A] + \frac{[S]}{K_m} + K_m + [S]} + K_m + [S] = \frac{K_m}{k_1} \frac{V}{[A]} (K_m + [S]).
\]

The features of the straight lines in Figs 4–8 can be seen from the reciprocal form:

\[
\frac{1}{v} = \frac{1}{V} + \frac{K_m}{V} \cdot [S] + \frac{K_m}{V} \cdot [A] (K_m + [S]).
\]

If substrate and activator are bound independently of each other, straight lines with a common point of intersection on the abscissa appear in the plots of \(1/v = f(1/[S])_A\) and \(1/v = f(1/[A])_S\).

For \([S] > K_m\) the quotient \(K_m/[S]\) in Eqn (5) becomes small and the slope of the straight lines in the plots of \(1/v - 1/[A]\) may become independent from \([S]\), for \([A] > K_A\) the slope of the plots \(1/v = f(1/[S])\) becomes independent of \([A]\). This has been the case in our experiments. Thus we have obtained straight lines (Figs 4 and 5) which appear to be nearly parallel.

At higher concentrations of divalent cations an inhibition of the E. coli ATPase occurs. The common point of intersection of the straight lines on the ordinate in the plot of \(1/v - 1/[S]\) (Fig. 7) and the common point of intersection in the second quadrant of \(1/v = f([Me])\) (Fig. 6) show, that Mg\(^{2+}\) ions are competitive inhibitors with respect to MgATP and Ca\(^{2+}\) ions with respect to CaATP (not shown). Thus Me\(^{2+}\) and [MeATP] probably compete for the substrate binding centre.

The competitive inhibition of E. coli ATPase by divalent metal ions explains the fact that other authors \(^{18-24}\) obtained higher \(K_m\) values than those reported in Table I. These authors determined the kinetic constants by variation of [MgATP] or [CaATP] at constant ratios [Me\(^{2+}\)]/[ATP] resulting in variable Me\(^{2+}\) concentrations and thus already inhibiting Mg\(^{2+}\) or Ca\(^{2+}\) ion concentrations. Our lower \(K_m\) values agree with the dissociation constant of the "loose" binding site which probably is the catalytic site of mitochondrial F\(_{1}\)-ATPase\(^{25}\). The activation and the competitive inhibition by Mg\(^{2+}\) explain the shift to lower pMg-values and the broadening of the pMg-optima in the \(v - pMe\)-plots with increasing substrate concentrations (Figs 1–3). In Figs 1–3 a shift of the Mg\(^{2+}\)-optimum to higher values can also be observed by increasing H\(^{+}\)-ion concentration. This may be explained by a competition between Mg\(^{2+}\) (or Ca\(^{2+}\)) and H\(^{+}\) ions for the metal ion binding centre.

There is no qualitative difference between Mg\(^{2+}\) or Ca\(^{2+}\) ions serving as activator. The kinetic constants and the enzyme activity differ somewhat. However, the exact difference between the action of Mg\(^{2+}\) and Ca\(^{2+}\) depends on the accuracy of the dissociation constants of MgATP and CaATP used. As the Ca\(^{2+}\) optima occur at higher concentrations than the Mg\(^{2+}\) optima, the latter metal ions are bound stronger than the Ca\(^{2+}\) ions.

From the dissociation equilibrium for MeATP it follows that varying [Me\(^{2+}\)] at constant [MeATP] also alters [ATP]. Therefore the above discussed effects of metal ions on E. coli ATPase might be considered being the result of an action of free [ATP] or of the combined action of [Mg\(^{2+}\)] and [ATP] as proposed in a recent paper by Skou\(^{26}\). The straight lines in Fig. 5 demonstrate that [ATP] had no influence. On the other hand, when \(1/v - 1/[MgATP]\) is plotted at constant [ATP] but variable [Mg\(^{2+}\)] (Fig. 9), one should expect straight lines if [ATP] but not [Mg\(^{2+}\)] influences ATPase activity. However, line [a] at 0.2 mM [ATP] is curved upwards as a result of the inhibitory effect of Mg\(^{2+}\) ions at high Mg\(^{2+}\) concentrations, [b] at 1.0 mM [ATP] is curved downwards because in
Fig. 9. Plot of $1/v$ vs $1/[\text{MgATP}]$ at different constant concentrations of free ATP. 100 mM Tris-HCl-buffer, pH 7.5.

1. $[\text{ATP}]: 0.2$ mM $(\times)$; 2. $[\text{ATP}]: 1.0$ mM $(\circ)$.

In this case the metal ion concentrations are lower and activating. Thus these results are in agreement with our assumption that Mg$^{2+}$ ions and not ATP affects the \textit{E. coli} ATPase.

Plotting $1/v - 1/[\text{ATP}]$ at constant [Me$^{2+}$] we obtained curved lines (not shown). On the other hand, plotting $1/v - 1[\text{MeATP}]$ (Figs 5 and 7) at constant [Me$^{2+}$] we obtained straight lines and thus MeATP fulfills Michaelis-Menten kinetics. Thus MeATP and not ATP is the actual substrate.

The competitive inhibition of the Mg-Ca-ATPase activity by Mg$^{2+}$ which appears at pMg 4 may have biological significance, as the intracellular pMg in \textit{E. coli} cells amounts to 3.27.

The Mg-Ca-ATPase of \textit{E. coli} consists of sub-units 19-20, 23-28. The ATPase-subunits $\alpha$ and $\gamma$ have ATPase-activity 29-30, $\delta$ is needed for membrane binding 29. The $\epsilon$-polypeptide could be the ATPase-inhibitor of the complete coupling factor and thus could have a regulatory function 29-30. Additionally activation and inhibition of the ATPase by Mg$^{2+}$ may also have a regulatory effect.

8. J. Lacy, Analyst 90, 65-75 [1965].