Effects of Mg\(^{2+}\), anions and cations on the Ca\(^{2+} + \text{Mg}^{2+}\)-activated ATPase of sarcoplasmic reticulum

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In a previous paper [Gould, East, Froud, McWhirter, Stefanova & Lee (1986) Biochem. J. 237, 217–227] we presented a kinetic model for the activity of the Ca\(^{2+} + \text{Mg}^{2+}\)-activated ATPase of sarcoplasmic reticulum. Here we extend the model to account for the effects on ATPase activity of Mg\(^{2+}\), cations and anions. We find that Mg\(^{2+}\) concentrations in the millimolar range inhibit ATPase activity, which we attribute to competition between Mg\(^{2+}\) and MgATP for binding to the nucleotide-binding site on the E1 and E2 conformations of the ATPase and on the phosphorylated forms of the ATPase. Competition is also suggested between Mg\(^{2+}\) and MgADP for binding to the phosphorylated form of the ATPase. ATPase activity is increased by low concentrations of K\(^+\), Na\(^+\) and NH\(_4\)\(^+\), but inhibited by higher concentrations. It is proposed that these effects follow from an increase in the rate of dephosphorylation but a decrease in the rate of the conformational transition E1'PCa\(_2\)–E2'PCa\(_2\) with increasing cation concentration. Li\(^+\) and choline\(^+\) decrease ATPase activity. Anions also decrease ATPase activity, the effects of I\(^-\) and SCN\(^-\) being more marked than that of Cl\(^-\). These effects are attributed to binding at the nucleotide-binding site, with a decrease in binding affinity and an increase in ‘off’ rate constant for the nucleotide.

INTRODUCTION

In a previous paper we presented a kinetic model for the Ca\(^{2+} + \text{Mg}^{2+}\)-activated ATPase (ATP phosphohydrolase, EC 3.6.1.3) purified from rabbit muscle sarcoplasmic reticulum (SR), which was consistent with the published data on the ATP- and pH-dependence of ATPase activity (Gould et al., 1986). Our aim is to combine this model with the model for Ca\(^{2+}\) efflux presented in the preceding paper (McWhirter et al., 1987) to describe both Ca\(^{2+}\) uptake and release in SR vesicles. However, this requires an extension of our previous model for ATPase activity to account for the complex effects for Mg\(^{2+}\), cations and anions.

We (Gould et al., 1986) have interpreted the kinetics of the ATPase in terms of two conformations for the ATPase, E1 and E2, as proposed by de Meis & Vianna (1979) (Scheme 1). In the E1 conformation, the Ca\(^{2+}\) - and MgATP-binding sites are of high affinity and are exposed to the outer (cytoplasmic) side of the SR membrane, whereas in the E2 conformation the sites are of low affinity and the Ca\(^{2+}\)-binding sites are exposed to the inside of the SR. The true substrate for the ATPase appears to be the MgATP complex rather than free ATP

Scheme 1. Reaction mechanism for the ATPase from Gould et al. (1986)

Abbreviations used: ATPase, Ca\(^{2+} + \text{Mg}^{2+}\)-activated ATPase; SR, sarcoplasmic reticulum.

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The experimental data show the ATPase activity as a function of $-\log[\text{ATP}]$ at $25^\circ C$, pH 7.2, $[K^+] = 12 \text{ mm}$ and $[Mg^{2+}] = 2.55 \text{ mm} (\bigcirc), 5.1 \text{ mm} (\square), 10.2 \text{ mm} (\bullet)$ and $20.4 \text{ mm} (\blacksquare)$. The lines are simulations with the parameters in Tables 1 and 2.

(Vianna, 1975; Gould et al., 1986). Highsmith (1984) has presented evidence for a bivalent-metal-ion-binding site at the MgATP-binding site, present in the absence of ATP. We have suggested that this is the binding site for Mg$^{2+}$ present on E2 and involved in phosphorylation by P$_i$ (Froud & Lee, 1986). Binding of MgATP to E1 and E2 will then be competitive with binding of Mg$^{2+}$ (Gould et al., 1986). To explain the dependence of ATPase activity on the concentration of MgATP, we suggested that MgATP also binds to the phosphorylated form of the ATPase, E1'PCa$_2$, and increases the rate of the transition to the E2'PCa$_4$ form (Gould et al., 1986). We showed that this effect could be simulated in terms of a single nucleotide-binding site, with MgATP binding to the site left vacant by the release of MgADP following phosphorylation of the ATPase. Since the concentration of free ATP will be negligible at the concentrations of Mg$^{2+}$ and ATP usually employed (typically 5 mm and 2 mm respectively), and if there is only one bivalent-metal-ion-binding site at the ATP-binding site, then this model requires that, following phosphorylation, MgADP is released from the ATPase rather than free ADP, so that MgATP can bind to the vacant site. Experiments on the phosphorylation of the ATPase by P$_i$ suggest that the affinity of the phosphorylated ATPase for Mg$^{2+}$ is high (Froud & Lee, 1986). Competition between binding of Mg$^{2+}$ and MgATP to the phosphorylated ATPase would then be expected, and it has been reported that high concentrations of Mg$^{2+}$ inhibit ATPase activity (Chiesi & Inesi, 1981; Andersen et al., 1985). In the present paper we investigate the effects of Mg$^{2+}$ in more detail and show how these effects can be included in the kinetic model for the ATPase.

It is known that a lysine residue lies close to the ATP-binding site of the ATPase, since labelling of lysine-515 with fluorescein isothiocyanate prevents binding of MgATP (Mitchinson et al., 1982; Brandl et al., 1986). Experiments with butanedione modification also suggest the presence of one or more arginine residues at the nucleotide-binding site (Murphy, 1976). Anion binding to $\epsilon$-amino groups is known in a number of enzymes, including alcohol dehydrogenase (Brändén et al., 1975), acetoacetate decarboxylase (Fridovich, 1972) and angiotensin-converting enzyme (Shapiro & Riordan, 1983). In alcohol dehydrogenase, it has been shown that Cl$^-$ increases the rate of dissociation of NADH (Brändén et al., 1975). Importantly, it has also been suggested that Cl$^-$ and other anions affect the binding of ADP to the phosphorylated ATPase (Shigekawa & Kanazawa, 1982). We have therefore studied anion effects in more detail.

Finally, it is known that K$^+$ increases the rate of dephosphorylation of the phosphorylated ATPase, but effects on the rate of the E1'PCa$_2$-E2'PCa$_4$ transition have also been suggested (de Meis, 1981). Effects of other cations on the activity of the ATPase have also been studied (The & Hasselbach, 1972; Shigekawa & Pearl, 1976; Duggan, 1977). In the present paper we study the specificity of these cation effects.

**MATERIALS AND METHODS**

Anal R reagents were obtained from BDH Chemicals, and Hepes (Ultrol) was from Calbiochem.

Ca$^{2+}$+Mg$^{2+}$-activated ATPase was prepared largely
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as described in East & Lee (1982). ATPase activity was determined at 25°C by using a coupled enzyme assay in a medium, unless otherwise specified, containing 40 mM-Hepes/KOH buffer, pH 7.2, 1.02 mM-EGTA, 0.92 mM-CaCl$_2$, 5 mM-MgSO$_4$, 2 mM-ATP, 0.42 mM-phosphoenolpyruvate, 0.15 mM-NADH, pyruvate kinase (7.5 units) and lactate dehydrogenase (18 units) in a total volume of 2.5 ml. The reaction was started by addition of the appropriate volume of 25 mM-CaCl$_2$ to a cuvette containing the ATPase and the other reagents. All buffers contained a minimum K$^+$ concentration of 12 mM, ensuring full activity of the coupling enzymes. Free Ca$^{2+}$ and Mg$^{2+}$ concentrations were calculated by using the binding constants given in Gould et al. (1986).

Kinetic simulations were carried out by using the FACSIMILE program (Chance et al., 1977) running on an ICL 2976 computer. For steady-state kinetics, equations were derived by using a version of the program KINAL of Cornish-Bowden (1977) modified to run on a Cromemco microcomputer, the simulations also being performed on the microcomputer.

RESULTS

ATPase activities were determined as a function of ATP concentration at maximally stimulating concentrations of Ca$^{2+}$. As shown in Fig. 1, the dependence of ATPase activity on the concentration of ATP is complex, with ATP at high concentrations producing a stimulation of activity. At all concentrations of ATP, increasing the concentration of Mg$^{2+}$ from 2.5 to 20.4 mM results in inhibition of ATPase activity (Fig. 1). The inhibitory effects of high concentrations of Mg$^{2+}$ were not reversed by increasing the concentration of Ca$^{2+}$ (results not shown). Activity is also seen to decrease with increasing concentration of K$^+$ (Fig. 2a); similar effects are seen with Na$^+$ and NH$_4^+$ (Figs. 2b and 2c).

As described elsewhere, ATPase activities for the purified ATPase are highly variable between preparations and also change on prolonged storage (Gould et al., 1986). ATPase activities for a preparation showing a higher ATPase activity are shown in Fig. 3(a) at fixed ATP and Mg$^{2+}$ concentrations of 2.1 mM and 5.1 mM respectively, as a function of added salt concentration. Activity is seen to increase slightly with increasing concentration of potassium methanesulphonate, compared with the slight decrease seen with KCl. In contrast, LiCl, choline chloride, KI and KSCN have marked inhibitory effects. As shown in Fig. 3(b), a rather similar pattern of effects is seen at low concentration (1 mM) of ATP, except that the inhibitory effect of LiCl is less
Table 1. Kinetic parameters for ATPase at 25 °C

Parameters were obtained by simulation as described in the text. Values for parameters not listed were taken from Gould et al. (1986). All unassigned rate constants are assumed to be high.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Equilibrium symbol</th>
<th>Value of constant</th>
<th>Forward rate constant (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_1'PCa_4 + Mg^{2+} \rightleftharpoons E_1'PMgCa_4$</td>
<td>$K_{23}$</td>
<td>$2 \times 10^{4}$*</td>
<td></td>
</tr>
<tr>
<td>$E_2'PCa_4 + Mg^{2+} \rightleftharpoons E_2'PMgCa_4$</td>
<td>$K_{23}$</td>
<td>$6 \times 10^{4}$†</td>
<td></td>
</tr>
<tr>
<td>$E_1'PCa_4 \rightleftharpoons E_2'PCa_4$</td>
<td>$K_{25}$</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>$E_1'PMgCa_4 \rightleftharpoons E_2'PMgCa_4$</td>
<td>$K_{24}$</td>
<td>0.03†</td>
<td></td>
</tr>
<tr>
<td>$E_1'PMgHCa_4 \rightleftharpoons E_2'PMgHCa_4$</td>
<td>$K_{24}$</td>
<td>0.03†</td>
<td></td>
</tr>
<tr>
<td>$E_1'PMgATPCa_4 \rightleftharpoons E_2'PMgATPCa_4$</td>
<td>$K_{24}$</td>
<td>0.03†</td>
<td>48.0§</td>
</tr>
<tr>
<td>$E_1'PMgATPHCa_4 \rightleftharpoons E_2'PMgATPHCa_4$</td>
<td>$K_{25}$</td>
<td>0.04†</td>
<td></td>
</tr>
<tr>
<td>$E_1'PMgATPHCa_4 + H^+ \rightleftharpoons E_1'(PH)MgHCa_4$</td>
<td>$K_{26}$</td>
<td>1.77 $\times 10^{6}$†</td>
<td></td>
</tr>
<tr>
<td>$E_1'PMgHCa_4 + K^+ \rightleftharpoons E_1'(PK)MgHCa_4$</td>
<td>$K_{27}$</td>
<td>24.4‡</td>
<td></td>
</tr>
<tr>
<td>$E_1'(PH)MgCa_4 \rightleftharpoons E_2'(PH)MgHCa_4$</td>
<td>$K_{24}$</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>$E_1'(PK)MgCa_4 \rightleftharpoons E_2'(PK)MgHCa_4$</td>
<td>$K_{24}$</td>
<td>0.03</td>
<td>9.6§</td>
</tr>
<tr>
<td>$E_1'(PH)MgATPCa_4 \rightleftharpoons E_2'(PH)MgATPCa_4$</td>
<td>$K_{25}$</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>$E_1'(PH)MgATPHCa_4 \rightleftharpoons E_2'(PH)MgATPHCa_4$</td>
<td>$K_{25}$</td>
<td>0.04</td>
<td>38.4§</td>
</tr>
<tr>
<td>$E_2 + MgATP \rightleftharpoons E_2MgATP$</td>
<td>$K_{12}$</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>$E_2 + Mg \rightleftharpoons E_1Mg$</td>
<td>$K_{18}$</td>
<td>110.0†</td>
<td></td>
</tr>
<tr>
<td>$E_2 + Mg \rightleftharpoons E_2Mg$</td>
<td>$K_{18}$</td>
<td>110.0†</td>
<td></td>
</tr>
<tr>
<td>$E_2Mg \rightleftharpoons E_1Mg$</td>
<td>$K_{1}$</td>
<td>2.5†</td>
<td>140†</td>
</tr>
<tr>
<td>$E_2 \rightleftharpoons E_1$</td>
<td>$K_{1}$</td>
<td>2.5†</td>
<td></td>
</tr>
<tr>
<td>$HE_2 \rightleftharpoons HE_1$</td>
<td>$K_{1}$</td>
<td>9.6§</td>
<td></td>
</tr>
<tr>
<td>$H_2E_2 \rightleftharpoons H_2E_1$</td>
<td>$K_{1}$</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>$E_1 + MgADP \rightleftharpoons E_1MgADP$</td>
<td>$K_{10}$</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>$E_1 + MgADP \rightleftharpoons E_1MgADP$</td>
<td>$K_{10}$</td>
<td>2.5 $\times 10^{4}$*</td>
<td></td>
</tr>
<tr>
<td>$E_1'PCa_4 + MgADP \rightleftharpoons E_1'PMgADPCa_4$</td>
<td>$K_{25}$</td>
<td>2.5 $\times 10^{4}$*</td>
<td></td>
</tr>
<tr>
<td>$E_2'PCa_4 + MgADP \rightleftharpoons E_2'PMgADPCa_4$</td>
<td>$K_{25}$</td>
<td>2.5 $\times 10^{4}$*</td>
<td></td>
</tr>
</tbody>
</table>

* Calculated from $K_{23}$, $K_{24}$, and $K_{26}$ in Froud & Lee (1986). Note that in Table 2 of Froud & Lee (1986) the value of $K_{26}$ was given incorrectly as 0.03.
† From Froud & Lee (1986) and Gould et al. (1986).
‡ Rate undefined, but could be low (see the text).
§ Varies between preparations (see the text).
¶ Constants for binding of H⁺ and K⁺ to the phosphogroup calculated from the constants in Froud & Lee (1986).
||| Values for KCl at 100 mm.

marked. The effects of LiCl and KSCN on the activity of the ATPase as a function of ATP concentration are shown in Fig. 4.

DISCUSSION

We have studied the effects of various ions on the activity of the Ca²⁺ + Mg²⁺-activated ATPase purified from rabbit muscle sarcoplasmic reticulum and present as membrane fragments in which ATPase activity is fully uncoupled from Ca²⁺ accumulation. The results show that concentrations of Mg²⁺ in excess of those necessary to convert free ATP into the MgATP complex inhibit ATPase activity (Fig. 1). The effect is not reversed by increasing Ca²⁺ concentrations and so cannot be attributed simply to competition between Mg²⁺ and Ca²⁺ for binding to the Ca²⁺-binding sites on the ATPase. To explain the complex dependence of ATPase activity on MgATP concentration, we have suggested that MgATP can bind both to the MgATP-binding site on the E1 form of the ATPase and to the empty nucleotide-binding site on the phosphorylated form E1'PCa₄, increasing the rate of the transition to E2'PCa₄ (Scheme 1, Gould et al., 1986). We have suggested previously that binding of Mg²⁺ to E1 is competitive with binding of MgATP. The inhibitory effects of Mg²⁺ seen at both high and low concentrations of MgATP suggest that binding of MgATP to E1'PCa₄ may also be competitive with binding of Mg²⁺.

The results shown in Fig. 3 indicate that anions also inhibit ATPase activity at both high and low concentrations of MgATP. The differences between the effects of increasing concentrations of potassium methanesulphonate, KCl, KI and KSCN indicate that effects of anions are specific and do not follow from a simple ionic-strength effect. Highsmith (1986) has reported that KI has no effect on ATPase activity up to 40 mm; the reason for this discrepancy is not known. The comparable effects of Mg²⁺ and anions on ATPase activity (Figs. 1, 3 and 4b) suggest that anions could also inhibit binding of MgATP to E1 and E1'PCa₄. The relative effects of the anions fall into what is commonly referred to as the ‘Hofmeister’ or lyotropic series, as has been found, for example, for the effects of anions on the activity of acetocetate decarboxylase (Fridovich, 1963). Finally, the results shown in Fig. 2 show that the K⁺, Na⁺ and NH₄⁺ ions have very similar effects on ATPase activity, whereas chloride salts of Li⁺ and choline⁺ are significantly more inhibitory than KCl (Fig. 3).

It is therefore necessary to extend the model for
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Table 2. Parameters for binding of anions to the ATPase at 25°C

<table>
<thead>
<tr>
<th>Anion</th>
<th>Equilibrium constant (I- or SCN-)</th>
<th>Value</th>
<th>Forward rate constant (s⁻¹)</th>
<th>Forward rate constant (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I⁻</td>
<td>Kᵣ</td>
<td>1.9 × 10⁴</td>
<td>1.8 × 10⁴</td>
<td>1.3 × 10⁴</td>
</tr>
<tr>
<td>SCN⁻</td>
<td>Kᵣ</td>
<td>2.0 × 10⁴</td>
<td>2.0 × 10⁴</td>
<td>1.7 × 10⁴</td>
</tr>
</tbody>
</table>

Fig. 5. Rate of phosphorylation of ATPase

The experimental data from Takisawa & Tonomura (1978) show the extent of phosphorylation (nmol/mg of protein) of the ATPase as a function of time either following additions of ATP to ATPase incubated with Ca²⁺ (○) or following simultaneous additions of Ca²⁺ and ATP (△). In both cases the final medium contained 100 mM-KCl, 1 mM-Mg⁺, 10 µM-ATP and 50 µM-Ca²⁺, pH 7.0. The curves are simulations with the parameters in Table 1 assuming an ATPase purity of 50%, but with an 'off' rate constant of 37 s⁻¹ and an association constant of 5 × 10⁶ for MgATP.

ATPase activity presented previously (Gould et al., 1986) to account for these effects of Mg²⁺, anions and cations.

It has been shown that the ATPase in the E₂ form can be phosphorylated by Pᵢ in the presence of Mg²⁺ (de Meis, 1981). We have suggested that the binding site for Mg²⁺ on E₂ involved in this phosphorylation is also present on E₁, and is the bivalent-metal-ion-binding site at the MgATP-binding site, so that binding of Mg²⁺ and MgATP will be competitive on E₁ (Gould et al., 1986). In Froud & Lee (1986) it was shown that binding constants for Mg²⁺ to E₁PC₃ and E₂PC₃ could be estimated from experiments on the phosphorylation by Pᵢ of SR vesicles passively loaded with Ca²⁺. The simplest explanation for the inhibition of ATPase activity by Mg²⁺ seen at high concentrations of MgATP is then that the binding of Mg²⁺ and of MgATP to E₁PC₃ and E₂PC₃ are also competitive. In the preceding paper (McWhirter et al., 1987) it was suggested that binding of Mg²⁺ to the non-phosphorylated forms of the ATPase inhibited the conversion of the ATPase between forms with inward-facing and outward-facing Ca²⁺-binding sites. Making the same assumption here, it is possible to fit the observed effects of Mg²⁺ on ATPase activity (Fig. 1) with the parameters given in Table 1. The binding constants for Mg²⁺ are those given previously in Froud & Lee (1986), but the binding constants of MgATP to the Mg²⁺-free E₁PC₃ and E₂PC₃ have been increased considerably over those given in Gould et al. (1986) (where competition between Mg²⁺ and MgATP was not considered), so that appreciable amounts of MgATP will be bound to E₁PC₃ and E₂PC₃ in the presence of Mg²⁺.

The inhibition of ATPase activity by anions at both high and low concentrations of MgATP is clearly not a non-specific ionic-strength effect, but can be simulated (Figs. 3 and 4) by assuming binding of anions at the
nucleotide-binding site, this binding resulting both in a
decreased affinity for MgATP and an increase in the ‘off’
rate constant for dissociation of MgATP from the
ATPase (Table 2). Cl\(^-\) binding to alcohol dehydrogenase
has been reported to increase the rate of dissociation of
NADH (Brändén et al., 1975), and it has been suggested by
Shigekawa & Kanazawa (1982) that binding of anions
to the phosphorylated ATPase increases the rate of
dissociation of ADP. An effect of anions on the rate of
dissociation of nucleotides requires, of course, the
simultaneous binding of the anion and nucleotide to the
protein, as indicated in Table 2. The greater effects of I\(^-\)
and SCN\(^-\) than of Cl\(^-\) can be simulated assuming equal
effects of the bound anions on the binding of MgATP,
but with the binding of I\(^-\) and SCN\(^-\) being stronger than
that of Cl\(^-\). The binding of the methanesulphonate anion
is too weak to have any significant effect (Fig. 3). This
order of effects would be consistent with binding at an
\(\varepsilon\)-amino group at the nucleotide-binding site, and a
number of such groups have been suggested to be
present (Murphy, 1976; Mitchinson et al., 1982; Brandl
et al., 1986), some of which are presumably involved
in interaction with nucleotide phosphate groups.

Simulations of the dependence of ATPase activity on
MgATP concentration are sensitive to the assumed ‘on’
rate constant for binding of MgATP to E1-PcA\(_2\), but
relatively insensitive to the ‘off’ rate constant if this is
low. A value for the ‘off’ rate constant (and hence for the
binding constant) can, however, be estimated from
experiments in which the extent of phosphorylation of
the ATPase is measured in mixtures of ATP and ADP,
as described below. The equilibrium constants for
binding of MgATP in the presence of bound anions given
in Table 2 are the maximum values consistent with the
data; equally good simulations are obtained with smaller
values.

As discussed elsewhere (Gould et al., 1986), there is
considerable uncertainty as to the correct binding
constant for MgATP to the ATPase. From direct
binding studies, dissociation constants of 2–3 \(\mu\)M
(Dupont, 1977; Möller et al., 1980), 4 \(\mu\)M (Meissner,
1973; Dupont et al., 1982), 5–6 \(\mu\)M (Andersen et al.,
1982), 7–8 \(\mu\)M (Nakamura & Tonomura, 1982b) and
16 \(\mu\)M (Guillain et al., 1984) have been reported. Since
these studies were performed with a variety of buffer
systems, temperatures and either detergent-purified
ATPase or SR vesicles, it is not possible to decide on
the reason for the variability. However, the possibility
of interaction of buffer ions with charged residues at the
binding site for MgATP deserves consideration.

The binding constant for MgATP must also be
consistent with data on the rate of phosphorylation of
the ATPase observed when low concentrations of
MgATP are added to the ATPase incubated in the
presence of Ca\(^{2+}\). Simulation (Fig. 5) of the rate of
phosphorylation observed by Takeda & Tonomura
(1978) with an MgATP concentration of 10 \(\mu\)M requires
an ‘on’ rate constant for binding of approx. \(2 \times 10^5\),
which, combined with an ‘off’ rate constant of 37 s\(^{-1}\),
gives an association constant of \(5 \times 10^4\). However, such
a binding constant predicts a higher rate of phosphorylation
than that observed experimentally by Fernandez-
Belda et al. (1984), also at 10 \(\mu\)M-MgATP (Fig. 6). The
data of Fernandez-Belda et al. (1984) can, however, be
simulated by assuming an association constant for
MgATP of \(2.5 \times 10^6\) (Fig. 6). As shown in Figs. 5 and 6,
the rate of phosphorylation observed on simultaneous
addition of MgATP and Ca\(^{2+}\) to ATPase is considerably
lower than that observed when MgATP is added to the
ATPase preincubated with Ca\(^{2+}\). The data of Fernandez-
Belda et al. (1984) are reproduced reasonably well by
using the parameters in Tables 1 and 2 and follow from
the relatively low rate of the E1–E2 transition.

As shown in the simulations, the extent of phosphoryl-
ation reached at long times after the initiation of
phosphorylation should be independent of the order in
which the components were mixed. This is not so in the
data reported by Takisawa & Tonomura (1978), and
the lower than expected extent of phosphorylation reached
on simultaneous addition of Ca\(^{2+}\) and ATP to the
ATPase incubated in EGTA can, perhaps, be attributed
to the reported relative instability of the ATPase in the
absence of Ca\(^{2+}\) (de Meis, 1981).

The data in Figs. 2 and 3 also show that cations have
important effects on ATPase activity. It is known that K\(^+\)
at concentrations in the 100 mM range very markedly
stimulates the rate of dephosphorylation of the ATPase
(de Meis 1981; Froud & Lee, 1986). It has also been
reported that K\(^+\) stimulates ATPase activity at concen-
trations up to about 50 mM but that higher concen-
trations produce little further stimulation or inhibition
(Shigekawa & Pearl, 1976; Duggan, 1977; Fig. 3). The
smaller effects of K\(^+\) on the overall rate of the ATPase
than expected from the known effects of K\(^+\) on
dephosphorylation have been attributed to a decrease in
the rate of the E1-PcA\(_2\)=E2-PcA\(_2\) transition in the
presence of K\(^+\) (Shigekawa & Akowitz, 1979). This effect
can be simulated in terms of the model for phosphoryl-
ation of the ATPase presented in Froud & Lee (1986).
In Froud & Lee (1986) it was shown that data on the
phosphorylation of the ATPase by P\(_i\) as a function of pH
and K\(^+\) concentrations could be fitted by assuming

![Fig. 6. Rate of phosphorylation of ATPase](image)
Effects of ions on sarcoplasmic-reticulum ATPase

![Graph](image)

**Fig. 7. Effect of ADP on phosphorylation of the ATPase**

The experimental data from Pickart & Jencks (1984) show the fractional extent of phosphorylation ([EP]/[EP_max]) of the ATPase at pH 7.0, [K+] = 100 mM, [Mg^{2+}] = 5.0 mM as a function of the concentration of ATP at added ADP concentrations of 0.052 mM (△), 0.106 mM (○), 0.321 mM (□), 0.763 mM (△) and 1.3 mM (●). The curves are simulations with the parameters in Tables 1 and 2.

binding of H^+ and K^+ to the phosphorylated aspartate residue, with binding constants of 1.77 x 10^7 and 24.4 respectively, the binding constants being the same for E1'PCA and E2'PCA (Froud & Lee, 1986; see Table 1). If it is assumed that the rate of the transition between E1'PCA and E2'PCA is affected by binding of H^+ or K^+ to the phospho group, then the experimental data on the effect of K^+ on ATPase activity can be simulated (Figs. 2 and 3). We find the best fit to the experimental data assuming a zero rate for the E1'PCA-E2'PCA transition when the phospho group is protonated, and a rate for the K^+-bound form 20% of that of the non-protonated form (Table 1). The similarity between the effects of K^+, Na^+ and NH_4^+ (Fig. 2) suggests that these three cations bind equally well to the ATPase, and have equal effects on the rates of the E1'PCA-E2'PCA transition and on dephosphorylation. In contrast, Li^+ and choline^+ have relatively little effect on activity (Figs. 3 and 4), suggesting either little binding to the ATPase or that binding has little effect on activity. The experimental data are best simulated by assuming that these cations can decrease the binding of K^+ to the ATPase, with Li^+ having 5% of the effect of K^+ on dephosphorylation and choline^+ no effect (Figs. 3 and 4). It is not clear whether competition between K^+ and Li^+ and choline^+ follows from competitive binding or from non-specific ionic-strength effects.

As reported in Gould et al. (1986), the activity of the ATPase is found to vary markedly between preparations and with time of storage, and these effects can be simulated by assuming just changes in the rates of the E1'PCA-E2'PCA and E1'PMgATPCa-E2'PMgATPCa transitions (Table 1). It has been reported that modification of the ATPase with N-ethylmaleimide can prevent the E1'PCA-E2'PCA transition (Kawakita et al., 1980; Nakamura & Tonomura, 1982a), so that the variability in the rate of this transition could reflect different extents of oxidation of essential thiol groups in the ATPase.

To complete the interpretation of the effects of Mg^{2+} on the ATPase, it is necessary to consider the consequences of the competition between the binding of Mg^{2+} and MgADP to E1'PCA. Pickart & Jencks (1984) have studied the effect of ADP on the amount of phosphorylated ATPase produced from [γ-32P]ATP. As shown in Fig. 7, a reasonable agreement to their data can be obtained with a binding constant for MgADP to E1'PCA of 2.5 x 10^4 (Table 1). Sufficient data are not available to determine how this binding constant varies with anion concentration. Shigekawa & Kanazawa (1982) estimated a binding constant for MgADP to E1'PCA of 6.0 x 10^4 in 100 mM-KCl and 1 mM-Mg^{2+}, compared with the effective binding constant at this Mg^{2+} concentration of 1.3 x 10^4 calculated from the parameters in Table 1. Pickart & Jencks (1984) estimated the binding constant to be 1.4 x 10^5 in 100 mM-KCl and 5 mM-Mg^{2+}, compared with the effective binding constant at this Mg^{2+} concentration of 5 x 10^4 calculated from the parameters in Table 1. For binding of MgADP to E1'Ca, Pickart & Jenks (1984) estimated a binding constant of 1.2 x 10^4 at 5 mM-Mg^{2+}, compared with the effective binding constant of 1.3 x 10^4 again calculated from the parameters in Table 1. Møller et al. (1980) measured an effective binding constant of 5.5 x 10^4 for MgADP to E1 in the presence of 1 mM-Mg^{2+}, compared with an association constant of 5 x 10^5 for MgATP under the same conditions. Thus, although the absolute values of the association constants measured by Møller et al. (1980) for MgADP and MgATP are different to those derived here, their ratio is the same. Cable et al. (1985) have measured the binding of ATP to E1 and to the phosphorylated ATPase in the presence of 0.1 mM-Mg^{2+}, and found comparable binding in the two cases. Although a detailed comparison is not possible, the parameters given in Tables 1 and 2 suggest that the binding constants for MgATP to E1 and to E1'PCA differ by no more than a factor of 3-fold under these conditions. The parameters presented in Tables 1 and 2 are also in agreement with the data of Pickart & Jenks (1982) on the rate of dephosphorylation of the ATPase caused by addition of ADP. Competition between MgADP and MgATP for E1'PCA depends on the "off" rate constant assumed for dissociation of MgATP. That given in Table 1 is the minimum consistent with the data in Fig. 6: higher values are inconsistent with the data on ATPase activity.

At low concentrations of Mg^{2+}, activity increases with increasing concentrations of Mg^{2+} (Chiesi & Inesi, 1981; Andersen et al., 1985). In part this can be attributed to the formation of increasing concentrations of the MgATP complex with increasing Mg^{2+} concentration, the MgATP complex being the true substrate for the ATPase. The decay of the phosphorylated ATPase E2'P is also Mg^{2+}-sensitive, decay of the bivalent-ion-free form being slow (Wakabayashi & Shigekawa, 1984). Finally, the experiments of Shigekawa & Dougherty (1979) suggest that the rate of the transition E1'PCA-E2'PCA may be low for the Mg^{2+}-free forms, but sufficient data are not yet available to model accurately this possibility.

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