Biochem. J. (1986) 237, 217–227 (Printed in Great Britain)

A kinetic model for the $Ca^{2+} + Mg^{2+}$-activated ATPase of sarcoplasmic reticulum

Gwyn W. GOULD, J. Malcolm EAST, Rosemary J. FROUD, Jennifer M. McWHIRTER, Helena I. STEFANOVA and Anthony G. LEE

Department of Biochemistry, University of Southampton, Bassett Crescent East, Southampton SO9 3TU, U.K.

The $Ca^{2+} + Mg^{2+}$-activated ATPase of sarcoplasmic reticulum exhibits complex kinetics of activation with respect to ATP. ATPase activity is pH-dependent, with similar pH-activity profiles at high and low concentrations of ATP. Low concentrations of Ca^{2+} in the micromolar range activate the ATPase, whereas activity is inhibited by Ca^{2+} at millimolar concentrations. The pH-dependence of this Ca^{2+} inhibition and the effect of the detergent $C_{12}E_8$ (dodecyl octaethylene glycol monoether) on Ca^{2+} inhibition are similar to those observed on activation by low concentrations of Ca^{2+}. On the basis of these and other studies we present a kinetic model for the ATPase. The ATPase is postulated to exist in one of two conformations: a conformation (E1) of high affinity for Ca^{2+} and MgATP and a conformation (E2) of low affinity for Ca^{2+} and MgATP. Ca^{2+} binding to E2 and to the phosphorylated form E2P are equal. Proton binding at the Ca^{2+}-binding sites in the E1 and E2 conformations explains the pH-dependence of Ca^{2+} effects. Binding of MgATP to the phosphorylated intermediate E1'PCa_2 and to E2 modulate the rates of the transport step E1'PCa_2–E2'PCa_2 and the return of the empty Ca^{2+} sites to the outside surface of the sarcoplasmic reticulum, as well as the rate of dephosphorylation of E2P. Only a single binding site for MgATP is postulated.

INTRODUCTION

Considerable effort has been invested in the study of the effects of the physical state (fluidity) and chemical structure of membrane phospholipids on the activities of membrane proteins. In order to understand the observed effects in molecular terms, it is necessary to relate changes in enzyme activity to specific conformational changes on the membrane protein. This, of course, can only be achieved in terms of a complete kinetic model for the protein. For most membrane proteins there is too little available information to allow the development of such a kinetic model. However, many data are available for the $Ca^{2+} + Mg^{2+}$-activated ATPase (ATP phosphohydrolase, EC 3.6.1.3) purified from rabbit muscle sarcoplasmic reticulum (de Meis, 1981). In the preceding two papers (Froud & Lee, 1986*a*,*b*) we have presented models for the binding of Ca^{2+} to the ATPase and for the phosphorylation of the ATPase by free phosphate. Here we complete the model by considering phosphorylation by ATP.

MATERIALS AND METHODS

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

SR was prepared from female rabbit (New Zealand White) hindleg muscle as described in Froud & Lee (1986*a*). ATPase activity was determined by using a coupled enzyme assay in a medium, unless otherwise specified, containing 40 mM-Hepes/KOH buffer, pH 7.2, 5 mM-$MgSO_4$, 2 mM-ATP, 0.42 mM-PEP, 0.15 mM-NADH, pyruvate kinase (7.5 units) and lactate dehydrogenase (18 units) in a total volume of 2.5 ml, with $CaCl_2$ and EGTA added to give the required free concentration of Ca^{2+}. The reaction was started by addition of an appropriate volume of 25 mM-$CaCl_2$ to a cuvette containing the ATPase and the other reagents.

Free Ca^{2+} concentrations were calculated by using the binding constants for Mg^{2+} and Ca^{2+} to EGTA and ATP and for H^+ to ATP given by Fabiato & Fabiato (1978) and for H^+ to PEP given by Vianna (1975). The constants listed for proton equilibria by Fabiato & Fabiato (1978) are true thermodynamic constants, involving H^+ concentration rather than the H^+ activity that is measured by a pH electrode. Accordingly, we have used the pK values for EGTA given by Boyd *et al.* (1965), which are mixed constants, incorporating H^+ activity and EGTA concentration. The final set of binding constants (pK_a values) was as follows: Ca–$EGTA^{4-}$, 10.716; Ca–$EGTA^{3-}$, 5.33; Mg–$EGTA^{4-}$, 5.21; Mg–$EGTA^{3-}$, 3.37; H–$EGTA^{4-}$, 9.38; H–$HEGTA^{3-}$, 8.77; H–H_2EGTA^{2-}, 2.68; H–H_3EGTA^{-}, 3.0; Ca–ATP^{4-}, 3.98; Ca–$HATP^{3-}$, 1.80; Mg–ATP^{4-}, 4.32; Mg–$HATP^{3-}$, 2.74; H–ATP^{4-}, 6.95; H–$HATP^{3-}$, 4.05; Ca–PEP, 2.08; Mg–PEP, 2.26; H–PEP, 6.35.

For experiments involving high concentrations of Ca^{2+} (> 100 μM) EGTA was omitted from the buffers, which were prepared from high-purity reagents in AnalaR water (BDH Chemicals).

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 microcomputer, the simulations also being performed on a microcomputer.

Abbreviations used: ATPase, $Ca^{2+} + Mg^{2+}$-activated ATPase; SR, sarcoplasmic reticulum; PEP, phosphoenolpyruvate; $C_{12}E_8$, dodecyl octaethylene glycol monoether.

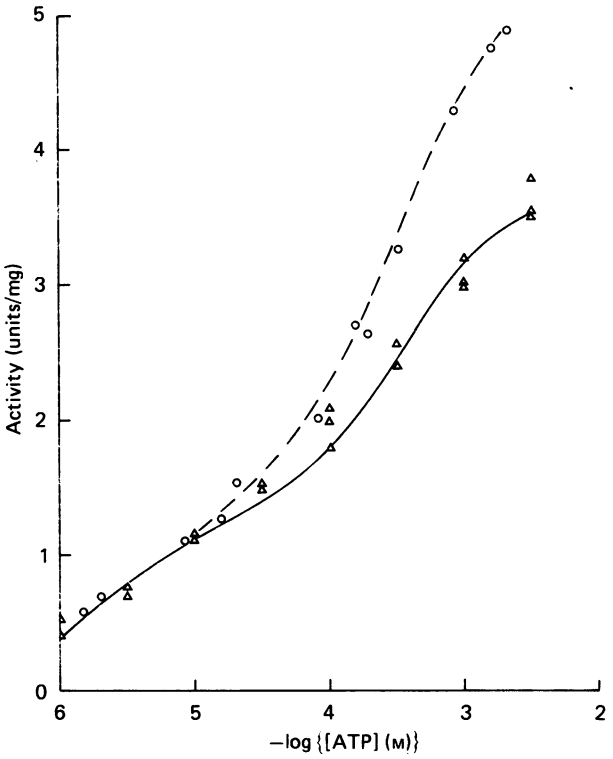


Fig. 1. ATPase activity as a function of ATP concentration

The experimental data show the ATPase activity as a function of $-\log[ATP]$ at 25 °C, pH 7.2, $[K^+] = 16$ mM and $[Mg^{2+}] = 5$ mM for a high-activity preparation (○) and low-activity preparation (△). The lines are simulations with the parameters in Table 1 and $k_{+5} = 86.2$ (——) or $k_{+5} = 47.1$ (——).

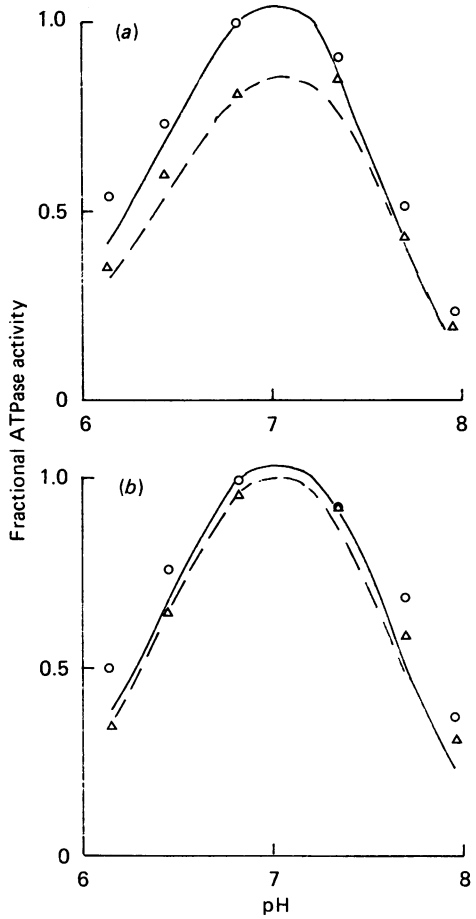


Fig. 2. ATPase activity as a function of pH and ATP concentration

The experimental data show the ATPase activity as a function of pH at 25 °C, $[Mg^{2+}] = 5$ mM and (*a*) [ATP] = 2107 μM or (*b*) [ATP] = 1.7 μM in the presence of 16 mM-KCl (△) or 116 mM-KCl (○), expressed relative to the activity at pH 7.2 and $[K^+] = 116$ mM. The lines are simulations with the parameters in Table 1 for $[K^+] = 116$ mM (——) and $[K^+] = 16$ mM (——).

RESULTS

ATPase activities measured at high (millimolar) ATP concentrations for the purified ATPase tend to be highly variable between preparations, without any differences in purity being obvious on SDS/polyacrylamide-gel electrophoresis. Fig. 1 shows the dependence of ATPase activity for a high-activity preparation and a low-activity preparation on the concentration of ATP. At low (micromolar) concentrations of ATP the activities of the two preparations are equal, showing that the low-activity preparation does not simply contain a large proportion of inactive protein, the difference in activities between the two preparations being most marked at high concentrations of ATP. The effect of pH at high and low concentrations of ATP is illustrated in Fig. 2, which also illustrates the more marked effect of K^+ on activity at high concentrations of ATP than at low concentration.

It is known that Ca^{2+} concentrations in the micromolar range activate the ATPase, whereas activity is inhibited by higher concentrations of Ca^{2+}. Figs. 3 and 4 illustrate the effects of high concentrations of Ca^{2+} as a function of pH and ATP concentration. At high Ca^{2+} concentrations EGTA is not a good buffer for Ca^{2+}, and it was therefore omitted for these experiments.

The detergent $C_{12}E_8$ has been shown to affect the binding of Ca^{2+} to the high-affinity site (Silva & Verjovski-Almeida, 1983). The effect of 0.5 mM-$C_{12}E_8$ on ATPase activity at high Ca^{2+} concentrations is shown in Fig. 5. Because of the reported instability of the ATPase in detergent in the absence of Ca^{2+}, for these experiments the ATPase was incubated for 10 min in buffers containing $C_{12}E_8$ and the required concentrations of Ca^{2+}, the reaction being initiated by the addition of ATP. The detergent was shown to have no effect on the coupling enzymes. Maximal ATPase activity (4.6 units/mg, pH 7.2, pCa = 4.5, ATP = 2107 μM, 25 °C) was unaffected by the addition of $C_{12}E_8$, but there was a clear shift in the inhibition curve to lower pCa values.

DISCUSSION

In a number of previous papers it has been reported that the dependence of the ATPase activity of the $Ca^{2+} + Mg^{2+}$-activated ATPase on the concentration of ATP is not simple, with ATP at high concentrations producing a stimulation of activity (Vianna, 1975; Dupont, 1977; Neet & Green, 1977; Taylor & Hattan,

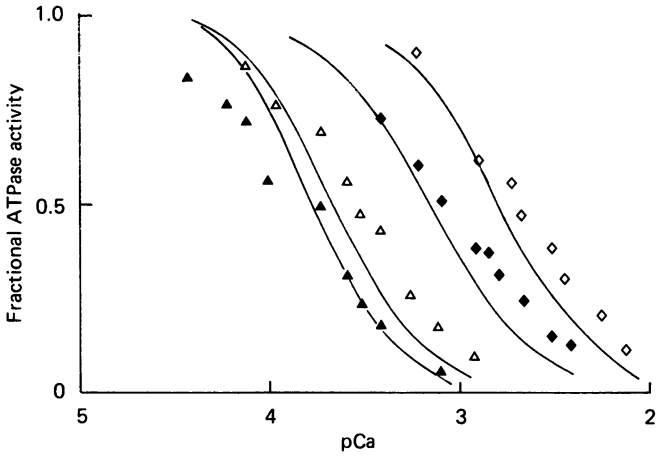


Fig. 3. Effect of high concentrations of Ca^{2+} on ATPase activity at 37 °C

The experimental data show the dependence of relative ATPase activity for the purified ATPase at 37 °C on pCa with $[K^+]$ = 16 mM, $[Mg^{2+}]$ = 5 mM, [ATP] = 2023 μM and pH 8.0 (▲), pH 7.2 (△), pH 6.5 (◆) and pH 6.1 (◇). The curves are simulations with the parameters in Tables 1 and 2.

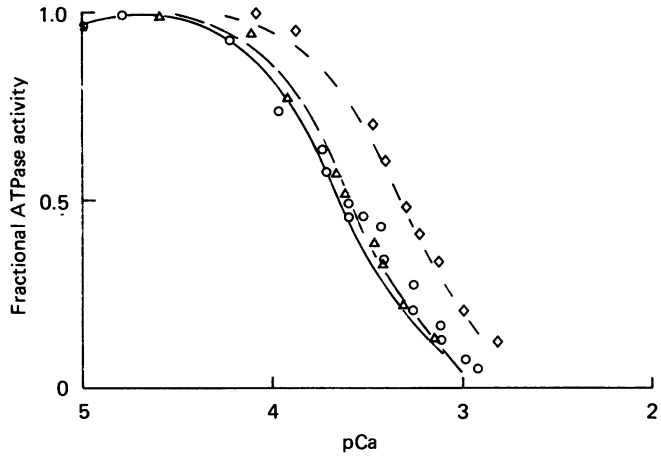


Fig. 4. Effect of high concentrations of Ca^{2+} on ATPase activity at 25 °C

The experimental data show the dependence of relative ATPase activity for the purified ATPase at 25 °C on pCa with $[K^+]$ = 16 mM, $[Mg^{2+}]$ = 5 mM, pH 7.2 and [ATP] = 2000 μM (○), 200 μM (△) and 2 μM (◇). The curves are simulations with the parameters in Tables 1 and 2.

1979; Anderson & Murphy, 1983; Kosk-Kosicka *et al.*, 1983). It has also been reported previously that ATPase activity measured at high concentrations of ATP is dependent on pH, with an optimum close to pH 7.2 (de Meis, 1981).

The experiment shown in Fig. 2 puts important constraints on any kinetic model for the ATPase: the effects of pH are very similar at both high and low concentrations of ATP. Fig. 2 also shows that K^+ has

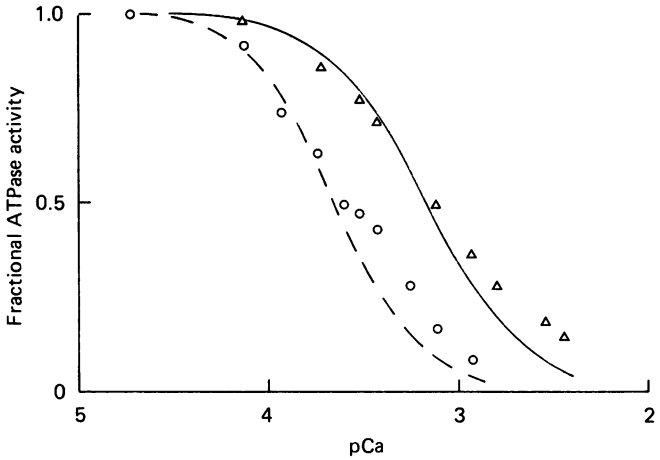


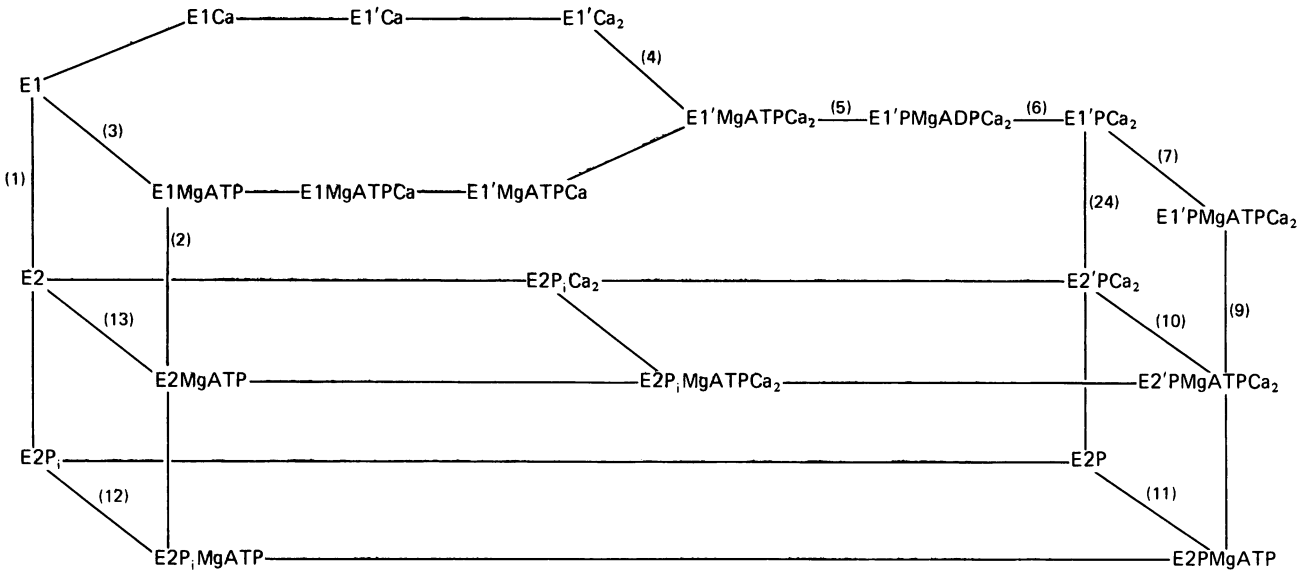
Fig. 5. Effect of $C_{12}E_8$ on ATPase activity

The experimental data show the relative ATPase activity of the purified ATPase at 25 °C in the absence (○) and in the presence (△) of 0.5 mM-$C_{12}E_8$, at pH 7.2 with $[K^+]$ = 16 mM, [ATP] = 2023 μM and $[Mg^{2+}]$ = 5 mM. The lines are simulations with the parameters in Tables 1 and 2 and K_{C5} = 0.08 (——) or K_{C5} = 1.1 (——).

only a relatively small effect on ATPase activity, particularly at low concentrations of ATP, despite the marked effect of K^+ on the rate of decay of phosphoenzyme (Froud & Lee, 1986*b*).

The only kinetic scheme that we could derive to account for these observations is shown in Scheme 1. It closely follows that proposed by de Meis (1981), except for the suggestion that the transition E1′PCa_2–E2′PCa_2 is affected by the binding of ATP. The ATPase is postulated to exist in two possible conformations E1 and E2 differing in affinities for Ca^{2+} and ATP. In the presence of micromolar concentrations of Ca^{2+}, the ATPase is phosphorylated by ATP (step 5) with the formation of a phosphoenzyme (step 6) that can react with ADP to produce ATP. A conformation change (steps 24 and 9) then converts the ADP-sensitive phosphoenzyme into an ADP-insensitive form in which the affinity for Ca^{2+} is low. Following release of Ca^{2+}, the enzyme is dephosphorylated and then undergoes a second conformational change to return to the E1 form (steps 1 and 2). The conformation changes E2–E1 and E1′PCa_2–E2′PCa_2 are postulated to be ATP-sensitive, as is the conformation change E1Ca–E1′Ca involved in binding of Ca^{2+} to the sites of high affinity (Froud & Lee, 1986*a*). We wish to stress that an effect of ATP on, for example, the rate of the E1–E2 transition is not an arbitrary supposition, but follows as a necessary consequence of a different binding constant of ATP for the two forms E1 and E2: the equilibrium constants E1/E2 and E1ATP/E2ATP will not be equal, and so the forward and back rates between E1 and E2 cannot equal those between E1ATP and E2ATP.

The binding of Ca^{2+} to ATPase in the absence of ATP has been shown to involve a slow conformation step E1Ca–E1′Ca (Froud & Lee, 1986*a*). Stahl & Jencks (1984) and Pickard & Jencks (1984) have measured the rate of phosphorylation of the ATPase following addition of Ca^{2+} to E1ATP, and shown it to be faster than



Scheme 1. Reaction mechanism for ATPase

the rate of the step E1Ca–E1'Ca. Their data are consistent with a rate constant for the conformation step of 180 s^{-1} in the presence of ATP compared with 12 s^{-1} in its absence (Fig. 6 and Table 1). The rate and equilibrium constants for the binding of Ca^{2+} in the absence of ATP have been defined in Froud & Lee (1986*a*). The dependence of ATPase activity on Ca^{2+} concentration at high concentrations of ATP (Figs. 6, 7 and 8) defines the equilibrium constants for the binding of Ca^{2+} in the presence of ATP. The data of Inesi & Hill (1983) are simulated well with the binding constant of Ca^{2+} to E1MgATP being 10-fold less than to E1, with the binding of Ca^{2+} to E1'MgATPCa being the same as in the absence of ATP. Watanabe *et al.* (1981) have shown that binding of Ca^{2+} to the ATPase is unaltered by the presence of the ATP analogue [$\alpha\beta$-imido]ATP, suggesting that the binding constant for ATP is the same to E1 and $E1'Ca_2$. The equilibrium constant for the transition E1Ca–E1'Ca must therefore be changed by the binding of ATP (Table 1). There are no data available on the rate of binding of Ca^{2+} to the ATPase in the presence of ATP, so for simplicity the 'on' rate constants have been put equal to those in the absence of ATP (Table 1).

The experimental free Ca^{2+} concentrations estimated in these experiments depend markedly on the values assumed for the EGTA–Ca^{2+} binding constant. Thus we find that with the binding constants we employ (see the Materials and methods section) the pCa value giving half-maximal activity at pH 7.2 is close to 6, both at 37 °C (Fig. 7) and at 25 °C (results not shown). Good agreement with the calculated activities based on the

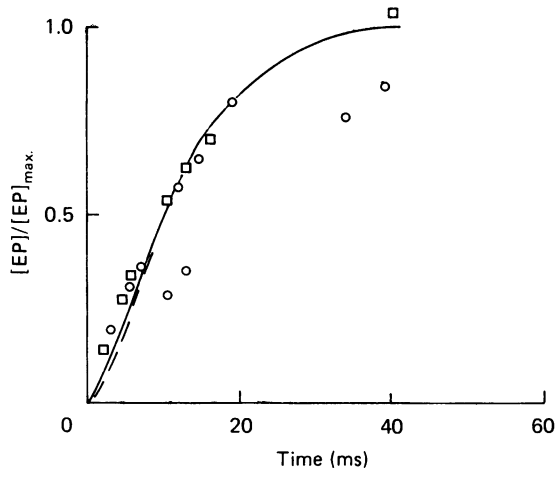


Fig. 6. Reaction of E1MgATP with Ca^{2+}

The experimental data from Stahl & Jencks (1984) show the fractional level of phosphorylation ([EP]/$[EP]_{max.}$) as a function of time following addition of 50 μM-Ca^{2+} (○) or 300 μM-Ca^{2+} (□) to ATPase incubated with 150 μM-ATP (○) or 100 μM-ATP (□) at pH 7 at 25 °C. The curves are simulations with the parameters in Table 1: ——, 300 μM-Ca^{2+}, 100 μM-ATP; ——, 50 μM-Ca^{2+}, 150 μM-ATP.

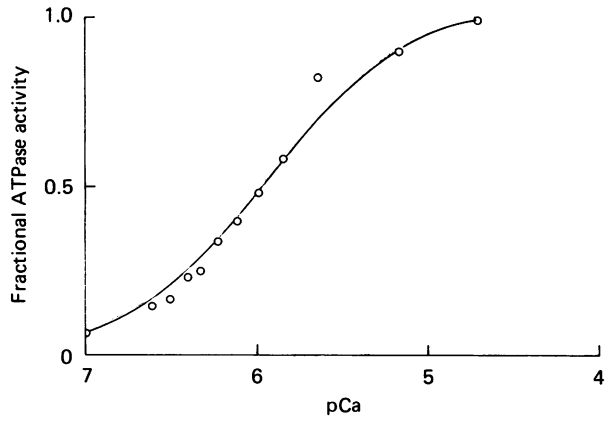


Fig. 7. ATPase activity as a function of Ca^{2+} concentration

The experimental data from Lee *et al.* (1983) show the dependence of relative ATPase activity for the purified ATPase at 37 °C on pCa at pH 7.2, $[K^+]$ = 16 mM, $[Mg^{2+}]$ = 5 mM and [ATP] = 2107 μM. The curves are a simulation with the parameters in Table 1.

Table 1. Kinetic parameters for ATPase at 25 °C

Parameters were obtained by simulation as described in the text. Parameters describing dephosphorylation are given in Table 1 of Froud & Lee (1986*b*). Rates of dephosphorylation of all Ca^{2+}-bound forms are assumed to be 0.05 times those of the Ca^{2+}-free form, and rates of dephosphorylation of all MgATP-free forms are assumed to be 1.5 times those of the MgATP-free forms. All unassigned rate constants are assumed to be fast.

Reaction	Equilibrium constant		Forward rate constant (s^{-1})
	Symbol	Value	
$E1+MgATP \rightleftharpoons E1MgATP$	K_3	1.25×10^5	18.5
$E1+Mg \rightleftharpoons E1Mg$	K_{16}	110.0	
$E1+MgADP \rightleftharpoons E1MgADP$	K_{14}	1.1×10^4	
$E1'Ca_2+MgATP \rightleftharpoons E1'MgATPCa_2$	K_4	1.25×10^5	45.5
$E1'Ca_2+Mg \rightleftharpoons E1'MgCa_2$	K_{16}	110.0	
$E1'Ca_2+MgADP \rightleftharpoons E1'MgADPCa_2$	K_{14}	1.1×10^4	
$E1MgATP+Ca^{2+} \rightleftharpoons E1MgATPCa$	K_{C7}	5.0×10^4	1.0×10^8
$E1MgATPH+Ca^{2+} \rightleftharpoons E1MgATPHCa$		*	1.0×10^8
$E1MgATPH_2+Ca^{2+} \rightleftharpoons E1MgATPH_2Ca$		*	1.0×10^8
$E1MgATPCa \rightleftharpoons E1'MgATPCa$	K_{C8}	16.8	180.0
$E1MgATPHCa \rightleftharpoons E1'MgATPHCa$		*	180.0
$E1MgATPH_2Ca \rightleftharpoons E1'MgATPH_2Ca$		*	180.0
$E1'MgATPCa+Ca^{2+} \rightleftharpoons E1'MgATPCa_2$	K_{C9}	1.44×10^8	1.0×10^8
$E1'MgATPHCa+Ca^{2+} \rightleftharpoons E1'MgATPHCa_2$		*	1.0×10^8
$E1'MgATPH_2Ca+Ca^{2+} \rightleftharpoons E1'MgATPH_2Ca_2$		*	1.0×10^8
$E1_0^{Ca}MgATP+H^+ \rightleftharpoons E1_0^{HCa}MgATP$†	K_{H9}	1.5×10^8	
$E1'^{Ca}_0MgATP+H^+ \rightleftharpoons E1'^{HCa}_0MgATP$†	K_{H10}	1.0×10^9	
$E1'MgATPCa_2+H^+ \rightleftharpoons E1'MgATPHCa_2$	K_{H8}	1.67×10^7	
$E1'MgATPCa_2 \rightleftharpoons E1'PMgADPCa_2$	K_5	0.5	500.0
$E1'PCa_2+MgADP \rightleftharpoons E1'PMgADPCa_2$	K_6	2.5×10^3	
$E1'PCa_2 \rightleftharpoons E2'PCa_2$	K_{24}	0.03	0
$E1'PHCa_2 \rightleftharpoons E2'PHCa_2$	K_{24}	0.03	0
$E1'PH_2Ca_2 \rightleftharpoons E2'PH_2Ca_2$	K_{24}	0.03	11.5
$E1'PCa_2+H^+ \rightleftharpoons E1'PHCa_2$‡	K_{H8}	1.67×10^7	
$E1'PHCa_2+H^+ \rightleftharpoons E1'PH_2Ca_2$‡	K_{H8}	1.67×10^7	
$E2'PCa_2+H^+ \rightleftharpoons E2'PHCa_2$‡	K_{H8}	1.67×10^7	
$E2'PHCa_2+H^+ \rightleftharpoons E2'PH_2Ca_2$‡	K_{H8}	1.67×10^7	
$E1'PCa_2+MgATP \rightleftharpoons E1'PMgATPCa_2$	K_7	2.2×10^3	3.3×10^6
$E1'PMgATPCa_2 \rightleftharpoons E2'PMgATPCa_2$	K_9	0.04	0
$E1'PMgATPHCa_2 \rightleftharpoons E2'PMgATPHCa_2$	K_9	0.04	0
$E1'PMgATPH_2Ca_2 \rightleftharpoons E2'PMgATPH_2Ca_2$	K_9	0.04	70.1§
$E2'PCa_2+MgATP \rightleftharpoons E2'PMgATPCa_2$	K_{10}	3.0×10^3‖	5.0×10^6
$E2+MgATP \rightleftharpoons E2MgATP$	K_{13}	2.0×10^4	
$E2MgATP \rightleftharpoons E1MgATP$	K_2	15.6¶	2000.0
$HE2MgATP \rightleftharpoons HE1MgATP$		**	221.4
$H_2E2MgATP \rightleftharpoons H_2E1MgATP$		**	24.3

* Defined by K_{H3}, K_{H8}, K_{H9} and K_{H10} (Table 2 in Froud & Lee, 1986*a*).
† 0 represents an unoccupied Ca^{2+}-binding site (see Froud & Lee, 1986*a*).
‡ Assumed to be unaffected by the binding of MgATP.
§ For a high-activity preparation; $k_{+9}=41.4\ s^{-1}$ for a low-activity preparation.
‖ Defined by K_7, K_{24} and K_9.
¶ Defined by K_1 (Table 1 in Froud & Lee, 1986*a*), K_3 and K_{13}.
** Defined by K_2, K_{H1} and K_{H2} (Table 1 in Froud & Lee, 1986*a*).

binding constants employed by Inesi & Hill (1983) is then achieved by shifting our pCa values by −0.27 unit. Equivalently, the binding constants K_{C1}, K_{C3} (Froud & Lee, 1986*a*), K_{C7} and K_{C9} can be altered to $K_{C1}=1\times10^6$, $K_{C3}=2.88\times10^8$, $K_{C7}=1\times10^5$ and $K_{C9}=2.88\times10^8$. We also find that the pCa values giving 50% activity vary significantly between preparations, with the higher-activity preparations generally having a lower pCa value for 50% activity (Rooney & Lee, 1983); this point is discussed further below.

Since at neutral pH in the presence of millimolar concentrations of Mg^{2+} essentially all the ATP present will be complexed with Mg^{2+}, it is likely that the true substrate of the ATPase is the MgATP complex rather than free ATP. There is considerable uncertainty as to the correct binding constant for MgATP to the ATPase. From direct binding studies Meissner (1973) has reported that binding is pH-dependent with a dissociation constant of 4 μM at pH 7, close to the values of 2–3 μM reported by Dupont (1977), 4 μM reported by Dupont

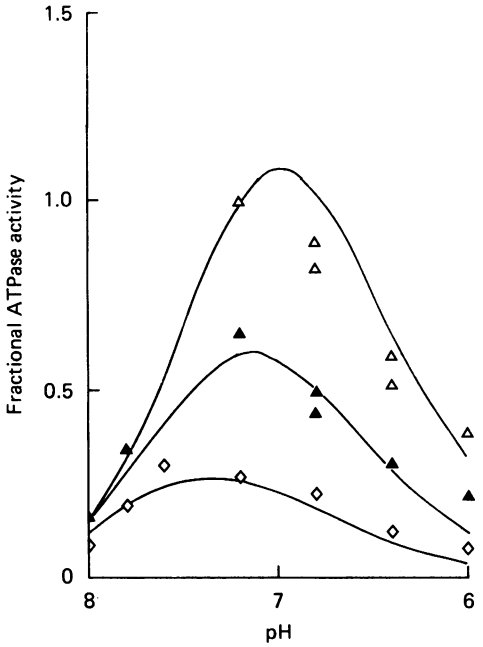


Fig. 8. ATPase activity as a function of pH and pCa

The experimental data from Inesi & Hill (1983) show the dependence of relative ATPase activity for leaky SR vesicles at 25 °C on pH with $[K^+] = 80$ mM, $[Mg^{2+}] = 10$ mM, [ATP] = 1 mM and pCa 5.5 (△), pCa 6.0 (▲) and pCa 6.5 (◇). The curves are simulations with the parameters in Table 1.

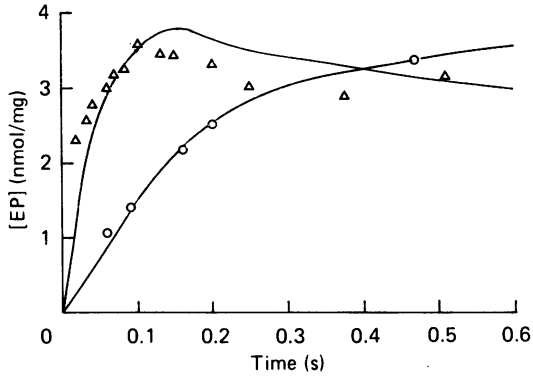


Fig. 9. Rate of phosphorylation of ATPase

The experimental data from Fernandez-Belda *et al.* (1984) show the level of phosphorylation (nmol/mg of protein) of the ATPase as a function of time either following additions of ATP to ATPase incubated with Ca^{2+} (△) or following simultaneous addition of Ca^{2+} and ATP (○). In both cases the final medium contained 80 mM-KCl, 10 mM-$MgCl_2$, 10 μM-ATP and 50 μM-Ca^{2+}, pH 6.8. The curves are simulations with the parameters in Table 1 and assuming an ATPase purity of 90% (△) and 75% (○) respectively. The decrease in phosphorylation level at long times in the simulation of the reaction initiated by addition of ATP follows from depletion of ATP.

et al. (1982) and 2.7 μM reported by Andersen & Møller (1985). Nakamura & Tonomura (1982) reported values of 7–8 μM, Andersen *et al.* (1982) values of 5–6 μM, Guillain *et al.* (1984) a value of 16 μM at pH 6 and Yates & Duance (1976) 40–50 μM. From indirect measurements, Pickart & Jencks (1984) estimated a binding constant of 20 μM. Although the binding of MgATP might be expected to be dependent on ionic strength, there is no obvious correlation between reported binding constants and ionic composition of media employed. We find the best fit to our kinetic data is obtained by assuming a dissociation constant of 8 μM (Table 1), whereas the best fit to some of the published data is obtained with a dissociation constant of 3 μM (see below). It seems possible therefore that the binding constant for MgATP varies between preparations of the ATPase.

Although we assume that the normal substrate is MgATP, in the absence of Mg^{2+} a CaATP complex will act as substrate (Wakabayashi & Shigekawa, 1984). Further, Highsmith (1984) has presented evidence for a bivalent-metal-ion-binding site at the ATP-binding site, present in the absence of ATP. Since we have proposed that the binding site for Mg^{2+} present on the E2 conformation of the ATPase and involved in phosphorylation by phosphate is also present on the E1 conformation (Froud & Lee, 1986*b*), it seems likely that this is the relatively non-specific bivalent-ion-binding site at the ATP-binding site. Binding of MgATP will then be competitive with binding of Mg^{2+}, providing a ready explanation for the inhibitory effects of high concentrations of Mg^{2+} (Ribeiro & Vianna, 1978; Andersen *et al.*, 1985).

Although the binding constant for MgATP appears to be the same for E1 and E1′Ca_2, it has been reported that the rates of dissociation of MgATP from E1 and E1′Ca_2 are different (Shigekawa & Kanazawa, 1982; Pickart & Jencks, 1982). Pickart & Jencks (1982) reported a dissociation rate constant for MgATP from E1′Ca_2 of 37 s^{-1} at 25 °C in the presence of 100 mM-KCl. Shigekawa & Kanazawa (1982) reported that the rate of dissociation of MgATP from E1′Ca_2 in the presence of 100 mM-KCl is twice that in its absence and that dissociation from E1 is about 3 times that from E1′Ca_2. Shigekawa & Kanazawa (1982) also reported that the effect of KCl is an anion effect, with SO_4^{2-} and SCN^- being more effective than Cl^-. The experiments of Pickart & Jencks (1982) can be simulated in terms of Scheme 1 with a rate constant for dissociation of 37 s^{-1} and a dissociation constant of 3 μM. We find the best fit to the steady-state measurements under our assay conditions with a dissociation rate of 18.5 s^{-1} (Fig. 1 and Table 1).

A lower limit to the rate of phosphorylation of E1′Ca_2 by ATP is given by experiments in which phosphorylation is initiated by addition of ATP to E1′Ca_2 (Verjovski-Almeida *et al.*, 1978; Inesi & Hill, 1983; Pickart & Jencks, 1984). Values for the rate constant of less than 300 s^{-1} give significantly lower rates than observed, maximal rates of phosphorylation being given by a rate constant of 500 s^{-1} or greater. The value of 500 s^{-1} has therefore been chosen (Table 1).

When phosphorylation is initiated by simultaneous addition of Ca^{2+} and ATP, the rate of phosphorylation is significantly lower than that observed following addition of ATP to E1′Ca_2, owing to the relatively low rate of both the E2–E1 transition and the E1Ca–E1′Ca transition. Fig. 9 illustrates a simulation of the data of

Fernandez-Belda *et al.* (1984) at pH 6.8 with the parameters in Table 1. Scofano *et al.* (1979) have shown that the difference between preincubation with Ca^{2+} and simultaneous addition of Ca^{2+} and ATP is more marked at pH 6 than at pH 7.4. Simulations show that this is because of the lower rate of the E2–E1 transition at pH 6 than at pH 7.4 (results not shown).

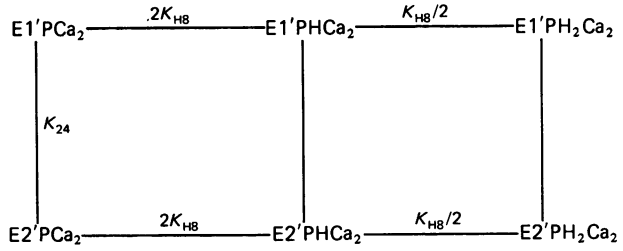
Following phosphorylation of the ATPase, ADP dissociates from $E1'PCa_2$. It is unclear whether this is in the form of free ADP or as a MgADP complex. Extensive studies with mitochondrial F_1-ATPases and kinases (see Gruys *et al.*, 1985) are consistent with the substrate being MgADP, and that provides the most straightforward explanation of the results for the ATPase. It has been suggested that free ADP dissociates from the ATPase leaving Mg^{2+} bound in an occluded form (Garrahan *et al.*, 1976). However, the reaction scheme that we propose involves the binding of ATP to the phosphorylated ATPase, and this would seem unlikely in the presence of occluded Mg^{2+}, since ATP will be present in the solution almost exclusively as MgATP and no evidence has been found for more than three bivalent-metal-ion-binding sites on the ATPase, the two Ca^{2+}-transport-binding sites and one at the ATP-binding site (Shigekawa *et al.*, 1983).

Pickart & Jencks (1982) and Froehlich & Heller (1985) have studied the effect of high concentrations of ADP on phosphorylation of the ATPase. The association constant of Mg^{2+} and ADP (Lemasters, 1984) is such that at neutral pH and excess Mg^{2+} ADP will be present predominantly as a MgADP complex. Assuming that this is the substrate for the back reaction, the data of Pickart & Jencks (1982) can be simulated assuming a value for the equilibrium constant for phosphorylation at pH 7 (K_5) of 0.5, a dissociation constant for MgATP of 3 μM and dissociation constants for MgADP from E1 and $E1'PCa_2$ of 90 μM and 0.4 mM respectively (results not shown) compared with values of 90 μM and 0.7 mM respectively derived by Pickart & Jencks (1982). Since we suggest below that MgATP binds similarly to $E1'PCa_2$ and $E2'PCa_2$ it is likely that MgADP binds equally to these two forms, but this does not affect the simulations. Fortunately, under the conditions of our studies in the presence of an ATP-regenerating system, the concentration of ADP will be very low, so that any uncertainty in the value of its binding constant will be unimportant in the simulations. We have found that increasing the concentrations of pyruvate kinase in the assay medium had no effect on ATPase activity, showing that residual concentrations of ADP are unimportant. Since the equilibrium constant for phosphorylation by phosphate is pH-dependent (Froud & Lee, 1986*b*), it is likely that K_5 will also be pH-dependent, but no experimental data seem to be available on this point.

Experiments on phosphorylation of the ATPase by phosphate in the presence of Ca^{2+} show that the affinity of $E1'PCa_2$ for Mg^{2+} must be high (Froud & Lee, 1986*b*). It would be expected, therefore, that, following dissociation of MgADP, Mg^{2+} would bind to $E1'PCa_2$. Our scheme for the reaction pathway includes binding of MgATP to $E1'PCa_2$, which, for the reasons described above, would have to be competitive with Mg^{2+} binding. In our experimental protocol, the total Mg^{2+} concentration is held constant at 5 mM, so that the free concentration of Mg^{2+} will decrease from 5 mM to approx. 3 mM as the ATP concentration is increased from 1 μM to 2000 μM (Fig. 1). However, we can best simulate the experimental dependence of ATPase activity on ATP concentration if this variation in Mg^{2+} concentration has little effect on the binding of MgATP to the phosphorylated ATPase. This implies that Mg^{2+} does not bind to $E1'PCa_2$ under the conditions of the experiment. A possible resolution of the problem is afforded by the observation by Wakabayashi & Shigekawa (1984) that binding of Mg^{2+} to the bivalent-cation-free phosphoenzyme is very slow at 0 °C. If the rate of binding of Mg^{2+} to $E1'PCa_2$ at 25 °C were low compared to the rate of the $E1'PCa_2$–$E2'PCa_2$ transition (and low compared with the rate of binding of MgATP), then it could be ignored under the conditions of the normal ATPase assay. However, if the concentration of $E1'PCa_2$ were to build up, then binding of Mg^{2+} to $E1'PCa_2$ could become important. It is therefore of interest that Nakamura (1984) found that at high Ca^{2+} concentrations (0.1–1 mM) decay of ^{32}P-labelled ATPase formed from [^{32}P]ATP following dilution with an excess of unlabelled ATP was biphasic, with a slow component whose proportion increased with time from initiation of the reaction: this could correspond to the formation of increasing amounts of $E1'PCa_2Mg$. Slow binding of Mg^{2+} to $E1'PCa_2$ could also explain the anomalous effects of ATP on the rate of decay of phosphorylated ATPase (Froud & Lee, 1986*b*). As described below, there is considerable evidence that decay of the phosphorylated ATPase formed from ATP is accelerated by ATP, whereas addition of ATP to the phosphorylated ATPase formed from Mg^{2+} and phosphate does not accelerate the decay of the phosphorylated ATPase (Froud & Lee, 1986*b*). When formed from phosphate, the phosphorylated enzyme will necessarily be present in an Mg^{2+}-bound form (E2PMg) with a high affinity for Mg^{2+}. Binding of MgATP will then be competitive with Mg^{2+}, and thus the effective binding constant for MgATP to the phosphorylated ATPase will be low, explaining the lack of effect of ATP.

There is now considerable evidence that dissociation of Ca^{2+} from $E1'PCa_2$ is very slow (Wakabayashi & Shigekawa, 1984): we have therefore put the rate to zero.

It is known that the rate of dephosphorylation of the phosphorylated form of the ATPase decreases with decreasing pH (see Froud & Lee, 1986*b*), as does the rate of the E2–E1 transition (from 3.3 at pH 6 to 23.2 at pH 7; Froud & Lee, 1986*a*), and steady-state ATPase activity also decreases from pH 7 to pH 6 (Fig. 2). Steady-state ATPase activity also decreases with increasing pH from pH 7 to pH 8 (Fig. 2). This effect must arise from a step following phosphorylation, since Inesi & Hill (1983) have shown that phosphorylation at high concentrations of ATP is fast at both pH 6.8 and pH 8.0. A possibility for a slow pH-sensitive step is the $E1'PCa_2$–$E2'PCa_2$ transition, and indeed Nakamura (1984) has provided evidence for a pH-sensitive step whose rate decreases between pH 6 and 8 and which could be this step. Simulations show that to obtain similar decreases in rate with increasing pH at both high and low concentrations of ATP (Fig. 2) it must be postulated that the rate of the $E1'PCa_2$–$E2'PCa_2$ step is modulated by binding of ATP, as has previously been postulated for the E2–E1 step. Studies by Nakamoto & Inesi (1984) and Bishop *et al.* (1984) with the ATP analogue trinitrocyclohexyldienyl-idine-ATP have indeed been interpreted in terms of binding of trinitrocyclohexyldienylidine-ATP to the ADP portion of the catalytic site following enzyme phosphorylation.



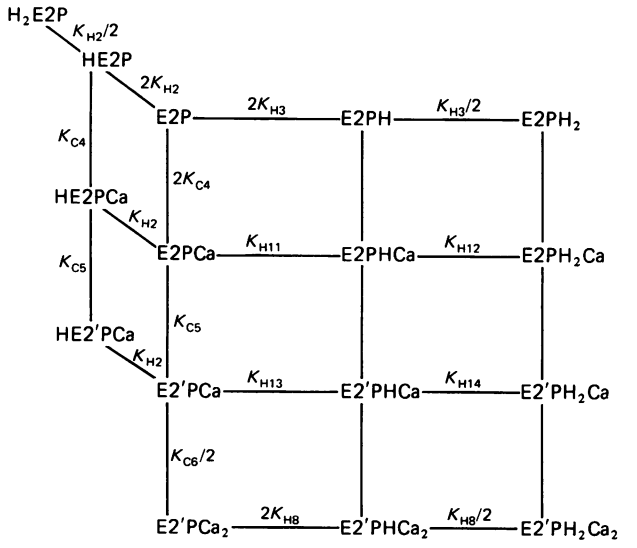
Scheme 2. Effect of pH on the E1'PCa$_2$–E2'PCa$_2$ transition

The observed decrease in rate with increasing pH requires the involvement of at least two protons. Variation of the rate of the E2–E1 transition with pH was also shown to involve two protons, and it was postulated that these were located at the two Ca^{2+}-binding sites (Froud & Lee, 1986*a*). A similar postulate is made here (Scheme 2). As described in Froud & Lee (1986*a*), each Ca^{2+}-binding site is postulated to contain two ionizable groups. Binding of Ca^{2+} is postulated to be competitive with protonation of one of these groups, but not with the other. To fit the pH-dependence of Ca^{2+} binding to the ATPase, the value of the proton-binding constant for the second residue for the site when occupied by Ca^{2+} (K_{H8}) was put equal to 1.67×10^7. Since there is no evidence that pH affects either the rate or extent of the initial steps of phosphorylation, we assign this same value to protonation of the residues at the Ca^{2+}-binding sites in the phosphorylated form E1'PCa$_2$ of the ATPase (Scheme 2). Further, phosphorylation of the ATPase by phosphate in the presence of Ca^{2+} can be simulated without assuming any pH-dependence of the E1'PCa$_2$–E2'PCa$_2$ transition. We therefore assign the same binding constant for protonation in the E2 form. Lastly, for simplicity we assume that binding of ATP does not affect protonation at the Ca^{2+}-binding sites. In terms of this scheme we can then successfully simulate the observed pH-dependence of ATPase activity (Figs. 2 and 8) with a dissociation constant for the binding of MgATP to the phosphorylated ATPase of 0.45 mM (Table 1), which can be compared with the value of 2.5 mM estimated by Watanabe & Inesi (1982) from the ability of MgATP to displace trinitrocyclohexyldienylidine-ATP. Since Bishop *et al.* (1984) found the rate of dissociation of trinitrocyclohexyldienylidine-ATP to be approx. 80-fold greater from E1'PCa$_2$ than from E1, we assign an 'off' rate constant of 1500 s^{-1} for the dissociation of ATP from E1'PCa$_2$ (Table 1). We find the best fit to the experimental data assuming that only the fully protonated form E1'PH$_2$Ca$_2$ can undergo the transition to the E2 form at an appreciable rate.

Over a number of years we have found that the activities of the ATPase vary widely between preparations and vary with time of storage at −20 °C, typical activities at 37 °C, pH 7.2, a saturating concentration of Ca^{2+} and an ATP concentration of 2 mM varying between about 14 and 20 units/mg. Fig. 1 shows the ATP-dependence of ATPase activity at 25 °C for a high-activity preparation and low-activity preparation. Activities are very similar at low concentration of ATP, showing that the low-activity preparation is not simply one containing a relatively high proportion of denatured ATPase. We find that differences in activities at high concentrations of ATP can be simulated simply assuming a different stimulation caused by ATP ($k_{+9} = 41.4$ for the low-activity preparation compared with $k_{+9} = 70.1$ for the high-activity preparation). These simulations also explain the different Ca^{2+}-dependences of ATPase activity for the high-activity and low-activity preparations. Since the E1'PCa$_2$–E2'PCa$_2$ transition is a slow step of the reaction sequence, an increase in the rate of this step will result in an apparent decrease in the affinity for Ca^{2+} as deduced from kinetic data, as observed (Rooney & Lee, 1983).

High concentrations of Ca^{2+} inhibit ATPase activity (Figs. 3 and 4), partly by reversing the steps from E2P to E1'PCa$_2$ and partly by inhibiting hydrolysis of E2P. Although it is possible to simulate the observed decrease in ATPase activity with increasing Ca^{2+} concentration purely by an effect on the steps from E2P to E1'PCa$_2$, the required values for the binding constant for Ca^{2+} to E2P and for the equilibrium constant E2'PCa$_2$/E1'PCa$_2$ are inconsistent with determinations of the phosphorylation of the ATPase by phosphate in the presence of Ca^{2+} (see Froud & Lee, 1986*b*). The data on phosphorylation by phosphate and on ATPase activity can both be fitted only if binding to E2P inhibits dephosphorylation. Data on the rate of phosphorylation by phosphate in the presence of Ca^{2+} were fitted assuming that the rates of phosphorylation and dephosphorylation of all forms of E2P with bound Ca^{2+} were 0.05 of the corresponding values for the Ca^{2+}-free form (Froud & Lee, 1986*b*). The same assumption is made here.

The dependence of ATPase activity on Ca^{2+} concentrations (Fig. 4) was used to determine the binding of Ca^{2+} to E2P. The scheme used to describe Ca^{2+} binding was exactly analogous to that used to describe Ca^{2+} binding to E1 (Scheme 3). We assume that the two Ca^{2+}-binding sites are identical and independent, so that the binding constants K_{H11}–K_{H14} can be expressed in a form



Scheme 3. Binding of Ca^{2+} to E2P

Binding of Ca^{2+} is competitive with protonation of residue 1 with proton association constant K_{H2}. Binding of Ca^{2+} is non-competitive with protonation of residue 2. For clarity, binding of protons to residue 1 is only indicated for the unprotonated state of residue 2.

exactly analogous to that used to describe binding to the E1 form (Froud & Lee, 1986*a*):

$$K_{H11} = K_{H3}+K_{H15}$$
$$K_{H12} = K_{H3}K_{H15}/(K_{H3}+K_{H15})$$
$$K_{H13} = K_{H8}+K_{H16}$$
$$K_{H14} = K_{H8}K_{H16}/(K_{H8}+K_{H16})$$

where K_{H15} and K_{H16} are binding constants for the protonation of residue 2 at the site occupied by Ca^{2+} in E2PCa and at the site unoccupied by Ca^{2+} in E2'PCa respectively. We find that the experimental data can be simulated with K_{H15} and K_{H16} having the same values as the equivalent constants in the scheme for proton binding to E1 (K_{H9} and K_{H10}). The variables to be fitted are then just those describing Ca^{2+} binding to E2, K_{C4}, K_{C5} and K_{C6} and the equilibrium constants K_{24} and K_9 for the steps E1'PCa_2–E2'PCa_2 and E1'PMgATPCa_2–E2'PMgATPCa_2 respectively. K_{24} is defined by experiments on the phosphorylation of the ATPase by phosphate in the presence of Ca^{2+} (Froud & Lee, 1986*b*), and K_9 is defined by the dependence of inhibition on the concentration of MgATP (Fig. 4). We find the best fit to the experimental data with $K_9 = 0.04$ compared with $K_{24} = 0.03$ (Table 1) with the Ca^{2+}-binding parameters given in Table 2. The slightly greater value of K_9 than of K_{24} implies a slightly stronger binding of MgATP to E2'PCa_2 than to E1'PCa_2. The parameters chosen provide a good fit to the pH-dependence (Fig. 3) and ATP-dependence (Fig. 4) of activity, and to the Ca^{2+}-dependence of phosphorylation by phosphate (Froud & Lee, 1986*b*). We have shown that the Ca^{2+}-dependence of phosphorylation by phosphate can be simulated assuming that Ca^{2+} binding to E2 and E2P are identical (Froud & Lee, 1986*b*), so that we describe binding to E2 by the same parameters used to describe binding to E2P. The simulations also assume that binding of ATP to E2P does not affect Ca^{2+} binding: this assumption could be relaxed, but would result in considerable further complexity in the rate equations, for which there is at present no requirement.

There are at present no firm data describing rates of Ca^{2+} binding to the E2 forms of the ATPase. These rates have therefore been described as fast (Table 2).

Table 2. Parameters describing the binding of Ca^{2+} to E2 and E2P

Parameters were obtained from simulation of the effects of high concentrations of Ca^{2+} on the ATPase activity.

Reaction	Equilibrium constant	
	Symbol	Value
$E2+H^+ \rightleftharpoons E2H$	K_{H3}	1.5×10^8
$E2'Ca_2+H^+ \rightleftharpoons E2'HCa_2$	K_{H8}	1.67×10^7
$E2_0^{Ca}+H^+ \rightleftharpoons E2_0^{HCa*}$	K_{H15}	1.5×10^8
$E2'_0{}^{Ca}+H^+ \rightleftharpoons E2'_0{}^{HCa*}$	K_{H16}	1.0×10^9
$E2+Ca^{2+} \rightleftharpoons E2Ca$	K_{C4}	2.2×10^3
$E2Ca \rightleftharpoons E2'Ca$	K_{C5}	1.1
$E2'Ca+Ca \rightleftharpoons E2'Ca_2$	K_{C6}	1.05×10^5

* 0 represents an unoccupied Ca^{2+}-binding site (see Froud & Lee, 1986*a*).

The parameters describing dephosphorylation in the absence of ATP have been derived in the preceding paper (Froud & Lee, 1986*b*). There is, however, considerable evidence that the rate of dephosphorylation of E2P is accelerated by binding of ATP (de Meis & de Mello, 1973; McIntosh & Boyer, 1983), McIntosh & Boyer (1983) estimating a stimulation of about 2-fold at millimolar concentrations of ATP. We find the best simulations assuming a stimulation of 1.5-fold (Table 1).

Rates of phosphorylation of the ATPase following addition of ATP and Ca^{2+} to Ca^{2+}-loaded SR vesicles define the rates of the E2–E1 transition. Comparison with rates of Ca^{2+} binding in the absence of ATP suggest that ATP accelerates the E2–E1 transition (Takisawa & Tonomura, 1978), with no effect on the pH-dependence of the transition (Scofano *et al.*, 1979). The simulations are relatively insensitive to the values for the binding constant of ATP to E2 and the rate of the E2ATP–E1ATP transition as long as the latter is high. The values given in Table 1 are the weakest binding constant and the lowest rate consistent with the data. We assume that the rate of the E2–E1 transition for any Ca^{2+}-bound forms is too low to be significant.

Finally, the equilibrium constants derived above should be consistent with the free energy of hydrolysis of ATP, since for a standard state of 1 M the Gibbs free-energy change for the cycle described in Scheme 1 is equal to the free energy of hydrolysis of ATP under the same conditions of pH, temperature and concentration of Mg^{2+}. At pH 7 and a Mg^{2+} concentration of 5 mM, the data of Lemasters (1984) give a standard free energy of hydrolysis $\Delta G^{0\prime}$ of between -28 and -30 kJ/mol. Under these same conditions the parameters derived above give a standard free-energy change for the cycle of -28.4 kJ/mol. At pH 6 the standard free-energy change for the cycle is -21 kJ/mol if it is assumed that K_5 is independent of pH, whereas that for the hydrolysis of ATP becomes between -26 and -28 kJ/mol. The simplest explanation for this discrepancy is that the equilibrium constant K_5 for phosphorylation is pH-dependent, and a value of 4.0 at pH 6 would give a standard free-energy change for the cycle of -26 kJ/mol.

Simulation of Ca^{2+} uptake by SR is not considered in detail here since it involves consideration of leak of Ca^{2+} from the SR vesicles, which is known to be pH- and ion-sensitive (J. McWhirter, G. Gould & A. G. Lee, unpublished work). However, if such leak is ignored, an approximate comparison can be made with literature data. Inesi & Hill (1983) and Inesi & Kurzmack (1983) have measured Ca^{2+} uptake as a function of time, quenching the reaction with acid and EGTA or EGTA and ADP. Under the former conditions it is likely that Ca^{2+} bound to all phosphorylated forms of the ATPase and Ca^{2+} trapped inside the SR will be measured, whereas under the latter conditions only Ca^{2+} bound to E2P and Ca^{2+} trapped inside the SR vesicles will be measured. Table 3 compares simulated time courses of Ca^{2+} uptake with the experimental data. At short times the experimentally measured Ca^{2+} uptake following quenching with EGTA shows less than two Ca^{2+} ions bound per molecule of phosphorylated ATPase, suggesting some loss of bound Ca^{2+} under these conditions. However, the difference between amounts of bound Ca^{2+} following EGTA and EGTA and ADP quenching are reproduced by the simulations at pH 6.0 and pH 6.8, and one set of measurements at pH 8 can be reproduced (Inesi &

Table 3. Comparison of observed and calculated Ca^{2+} uptake by SR vesicles

The experimental data describe Ca^{2+} uptake as a function of time following the addition of 1 mM-ATP to SR vesicles incubated with 50 μM-Ca^{2+} at the given pH and quenched with either EGTA or EGTA + ADP. The experimental values are taken from Inesi & Hill (1983) (*) and from Inesi & Kurzmack (1983) (†). The theoretical calculations were performed with the parameters in the Tables, assuming that 60% of the SR protein was active ATPase and ignoring any leak of Ca^{2+} from the SR vesicles.

		Ca^{2+} uptake (nmol/mg of protein)			
Conditions	Time ...	50 ms	100 ms	200 ms	300 ms
pH 6, EGTA	Exptl.*	5	7	12	13
	Calc.	10	11	14	16
pH 6, EGTA/ADP	Exptl.*	2–3	4	6	9
	Calc.	2.5	6	10	12
pH 6.8, EGTA	Exptl.*	5–9	8–9	14	16
	Calc.	10	12	14	16
pH 6.8, EGTA/ADP	Exptl.*	2–3	3–5	7	11
	Calc.	1	3	6	8
pH 8, EGTA	Exptl.*	4	4	4	4
	Exptl.†	5–9	9	9	8
	Calc.	11	11	11	11
pH 8, EGTA/ADP	Exptl.*	2	2	2	2
	Calc.	0.5	0.5	1	1

Kurzmack, 1983) although not the other (Inesi & Hill, 1983).

The set of parameters presented in this and the two preceding papers (Froud & Lee, 1986*a*,*b*) provide a basis for understanding at least most of the published data on the kinetics of the ATPase. It is difficult in an analysis such as this to assign an accuracy for each rate parameter, since many of these rates are interdependent in the overall reaction scheme for the ATPase, although studies of partial reactions serve to define many of them independently. In general, and unless otherwise stated, variation of any of the given rate parameters by more than 10–20% causes significantly worse fits between experimental and calculated data.

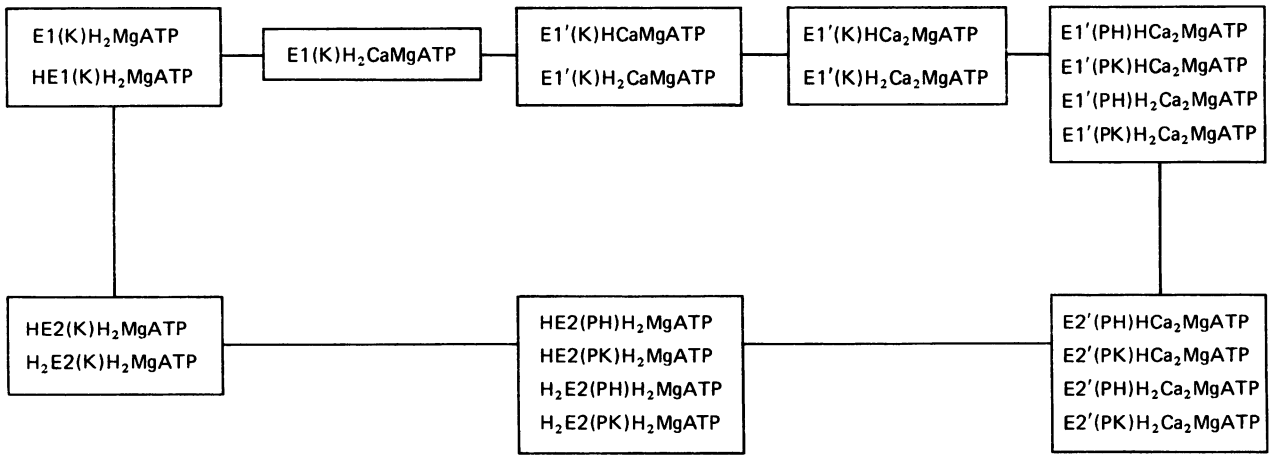
Scheme 4 represents the major species involved in the reaction cycle under conditions that can be considered physiological (5 mM-MgATP, pH 7, 100 mM-K^+, an external Ca^{2+} concentration sufficient to saturate the high-affinity Ca^{2+}-binding sites and an internal Ca^{2+} concentration low enough not to bind significantly to the low-affinity Ca^{2+} sites).

Although this is not the place to discuss at length the interpretation that should be placed on the parameters derived here, a few general comments are probably not out of place. First, the formation of an acyl phosphate from ATP bound at the active site:

$$E1'MgATPCa_2 \rightleftharpoons E1'PMgADPCa_2$$

occurs with an equilibrium constant close to unity ($K_5 = 0.5$). Further, although Gibbs free-energy changes for the various steps of Scheme 1 vary over a wide range at the standard state of 1 M, they are all close to zero under conditions typical of those in a muscle cell (8 mM-ATP, 0.9 mM-ADP, 8 mM-phosphate, 5 mM-Mg^{2+}, 0.1 μM external Ca^{2+}, 1 mM internal Ca^{2+}; Veech *et al.*, 1979). This prevents the accumulation of a large fraction of the enzyme in any one particular form and maximizes the rate of the reaction.

The step of the reaction cycle that can be said to be driven by ATP is the transport step, the conversion of $E1'PCa_2$ into $E2'PCa_2$. For the unphosphorylated ATPase the equilibrium constant $E1Ca_2/E2Ca_2$ is approx. 10^6 whereas for the phosphorylated ATPase the equilibrium constant $E1'PCa_2/E2'PCa_2$ has been reduced to 33 ($1/K_{24}$), thus allowing transport of Ca^{2+} across the membrane at a significant rate. It is this, together with slow dissociation of Ca^{2+} from $E1'PCa_2$, that, we believe, ensures tight coupling between ATP hydrolysis and Ca^{2+} uptake. It seems likely that, in principle, transitions can occur between all corresponding states of the E1 and E2



Scheme 4. Major species involved in ATPase activity

The major species involved in ATPase activity are shown under conditions similar to physiological conditions, with 5 mM-MgATP, pH 7, 100 mM-K^+, saturating external Ca^{2+} and low internal Ca^{2+} concentrations. (K) refers to K^+ bound at the phosphorylation site and (PH) and (PK) refer to bound H^+ and K^+ respectively at the phosphorylation site after phosphorylation. Binding of H^+ and Ca^{2+} to E1 and E2 is as described in the text.

conformations, but the stronger binding of MgATP and Ca^{2+} to the E1 conformation ensures that the only transitions to occur at a significant rate are E1'PCa_2–E2'PCa_2 and E2–E1, together with the corresponding transitions for the MgATP-bound forms.

The advantage of a two-conformation model of the type utilized here is that it provides a conceptually straightforward scheme for the transport event. Further, it is worth stressing that the model proposed here has involved just two identical Ca^{2+}-binding sites and one ATP-binding site, containing a nucleotide-binding region and the amino acid residue to be phosphorylated, with a monomeric active unit. Of course, since we have shown that the kinetics can be interpreted in terms of a single ATP-binding site, they are equally consistent with two ATP-binding sites (although the opposite case would not be true). Carvalho-Alves *et al.* (1985) have interpreted their photo-labelling experiments with ATP analogues in terms of distinct high-affinity and low-affinity ATP-binding sites, but their data are by no means conclusive. Froehlich & Heller (1985) have invoked a dimer model to explain the kinetics of the ATPase, but this at present seems unnecessary. Thus it has been shown that the ATPase is fully active in monomeric form dissolved in the detergent $C_{12}E_8$ (Kosk-Kosicka *et al.*, 1983). The kinetics of the ATPase are considerably different in $C_{12}E_8$ from those in a normal membrane environment, but the observed change in Ca^{2+}-dependence can be simulated by the change in E1Ca–E1'Ca and E2PCa–E2'PCa transitions described above and the observed change in ATP-dependence can be simulated by an increase in affinity of E1'PCa_2 for MgATP and an increased rate for the E1'PMgATPCa_2–E2'PMgATPCa_2 transition (results not shown).

We thank the Agricultural and Food Research Council, Science and Engineering Research Council, Muscular Dystrophy Group of Great Britain and the Wellcome Trust for financial support and the Medical Research Council for a studentship to G.W.G.). J.M.E. is a Wellcome Trust Lecturer.

REFERENCES

Andersen, J. P. & Møller, J. V. (1985) Biochim. Biophys. Acta **815**, 9–15
Andersen, J. P., Møller, J. V. & Jørgensen, P. L. (1982) J. Biol. Chem. **257**, 8300–8307
Andersen, J. P., Lassen, K. & Møller, J. V. (1985) J. Biol. Chem. **260**, 371–380
Anderson, K. W. & Murphy, A. J. (1983) J. Biol. Chem. **258**, 14276–14278
Bishop, J. E., Johnson, J. D. & Berman, M. C. (1984) J. Biol. Chem. **259**, 15163–15171
Boyd, S., Bryance, A., Nancollas, G. H. & Torrance, K. (1965) J. Chem. Soc. 7353–7358
Carvalho-Alves, P. C., Oliveira, C. R. G. & Verjovski-Almeida, S. (1985) J. Biol. Chem. **260**, 4282–4287
Chance, E. M., Curtis, A. R., Jones, I. P. & Kirby, C. R. (1977) FACSIMILE: A Computer Program for Flow and Chemistry Simulation, H.M.S.O., London
Cornish-Bowden, A. (1977) Biochem. J. **165**, 55–59
de Meis, L. (1981) The Sarcoplasmic Reticulum, pp. 1–163, John Wiley and Sons, New York
de Meis, L. & de Mello, M. C. F. (1973) J. Biol. Chem. **248**, 3691–3701
Dupont, Y. (1977) Eur. J. Biochem. **72**, 185–190
Dupont, Y., Chapron, Y. & Pougeois, R. (1982) Biochem. Biophys. Res. Commun. **106**, 1272–1279
Fabiato, A. & Fabiato, F. (1978) J. Physiol. (Paris) **75**, 463–505
Fernandez-Belda, F., Kurzmack, M. & Inesi, G. (1984) J. Biol. Chem. **259**, 9687–9698
Froehlich, J. P. & Heller, P. F. (1985) Biochemistry **24**, 126–136
Froud, R. J. & Lee, A. G. (1986*a*) Biochem. J. **237**, 197–206
Froud, R. J. & Lee, A. G. (1986*b*) Biochem. J. **237**, 207–215
Garrahan, P. J., Rega, A. F. & Alonso, G. L. (1976) Biochim. Biophys. Acta **448**, 121–132
Gruys, K. J., Urbauer, J. L. & Schuster, S. M. (1985) J. Biol. Chem. **260**, 6533–6540
Guillain, F., Champeil, P. & Boyer, P. D. (1984) Biochemistry **23**, 4754–4761
Highsmith, S. (1984) Biochem. Biophys. Res. Commun. **124**, 183–189
Inesi, G. & Hill, T. L. (1983) Biophys. J. **44**, 271–280
Inesi, G. & Kurzmack, M. (1983) in Biomembrane Structure and Function (Chapman, D., ed.), pp. 1355–1410, Macmillan, London
Kosk-Kosicka, D., Kurzmack, M. & Inesi, G. (1983) Biochemistry **22**, 2559–2567
Lee, A. G., East, J. M., Jones, O. T., McWhirter, J. M., Rooney, E. K. & Simmons, A. C. (1983) Biochim. Biophys. Acta **732**, 441–454
Lemasters, J. J. (1984) J. Biol. Chem. **259**, 13123–13130
McIntosh, D. B. & Boyer, P. D. (1983) Biochemistry **22**, 2867–2875
Meissner, G. (1973) Biochim. Biophys. Acta **298**, 906–926
Nakamoto, R. K. & Inesi, G. (1984) J. Biol. Chem. **259**, 2961–2970
Nakamura, Y. (1984) J. Biol. Chem. **259**, 8183–8189
Nakamura, Y. & Tonomura, Y. (1982) J. Bioenerg. Biomembr. **14**, 307–310
Neet, K. E. & Green, N. M. (1977) Arch. Biochem. Biophys. **178**, 588–597
Pickart, C. M. & Jencks, W. P. (1982) J. Biol. Chem. **257**, 5319–5322
Pickart, C. M. & Jencks, W. P. (1984) J. Biol. Chem. **259**, 1629–1643
Ribeiro, J. M. C. & Vianna, A. L. (1978) J. Biol. Chem. **253**, 3153–3157
Rooney, E. K. & Lee, A. G. (1983) Biochim. Biophys. Acta **732**, 428–440
Scofano, H. M., Viegra, A. & de Meis, L. (1979) J. Biol. Chem. **254**, 10227–10231
Shigekawa, M. & Kanazawa, T. (1982) J. Biol. Chem. **257**, 7657–7665
Shigekawa, M., Wakabayashi, S. & Nakamura, H. (1983) J. Biol. Chem. 14157–14161
Silva, J. L. & Verjovski-Almeida, S. (1983) Biochemistry **22**, 707–716
Stahl, N. & Jencks, W. P. (1984) Biochemistry **23**, 5389–5392
Takisawa, H. & Tonomura, Y. (1978) J. Biochem. (Tokyo) **83**, 1275–1284
Taylor, J. S. & Hattan, D. (1979) J. Biol. Chem. **254**, 4402–4407
Veech, R. L., Lawson, J. W. R., Cornell, N. W. & Krebz, H. A. (1979) J. Biol. Chem. **254**, 6538–6547
Verjovski-Almeida, S., Kurzmack, M. & Inesi, G. (1978) Biochemistry **17**, 5006–5013
Vianna, A. L. (1975) Biochim. Biophys. Acta **410**, 389–406
Wakabayashi, S. & Shigekawa, M. (1984) J. Biol. Chem. **259**, 4427–4436
Watanabe, T. & Inesi, G. (1982) J. Biol. Chem. **257**, 11510–11516
Watanabe, T., Lewis, D., Nakamoto, R., Kurzmack, M., Fronticelli, C. & Inesi, G. (1981) Biochemistry **20**, 6617–6625
Yates, D. W. & Duance, V. C. (1976) Biochem. J. **259**, 6617–6625

Received 7 November 1985/15 January 1986; accepted 7 March 1986