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Mechanistic Origin of the Kinetic Cooperativity for the ATPase Activity of Sarcoplasmic Reticulum[1]

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Abstract

The (Ca^{2+}-Mg^{2+})-ATPase from sarcoplasmic reticulum presents negative cooperativity for the hydrolysis of Mg^{2+}-ATP at different concentration ranges of this substrate. A kinetic model is proposed according to which Mg^{2+}-ATP may bind to three different enzymatic species present during the catalytic cycle, E ($K_1 = 1\,\mu M$), E' ~ P.Ca_2 ($K_9 = 500\,\mu M$) and *EP ($K_7 = 20\,\mu M$), accelerating the release of P_i. The fact that each of these species has a different affinity for Mg^{2+}-ATP allows a significant enhancement of the rate of P_i release to the medium at the different ranges of Mg^{2+}-ATP concentration where the enzyme shows a kinetic cooperativity. The kinetic analysis of this mechanism yields an equation which is a ratio of two cubic polynomials (3:3 rate equations) with respect to Mg^{2+}-ATP and which may explain the negative cooperativity of the enzyme at different concentration ranges of Mg^{2+}-ATP.

Key Words: (Ca^{2+}-Mg^{2+})-ATPase; sarcoplasmic reticulum; enzyme kinetics.

Introduction

It is well known that double-reciprocal plots of the ATPase activity from sarcoplasmic reticulum versus Mg^{2+}-ATP concentration show nonlinear dependence both at low (0.75–50 μM) Mg^{2+}-ATP concentrations (Yamamoto and Tonomura, 1967; Kazanawa *et al.*, 1971; Panet *et al.*, 1971; Vianna, 1975; Yates and Duance, 1978; Neet and Green, 1977; Möller *et al.*, 1980) and also at high (0.1–10 mM) Mg^{2+}-ATP concentrations (Yamamoto and

[1]Abbreviations: EGTA, ethylene glycol bis(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid; I.U., international units; piruvate kinase (EC 2.7.1.40); lactate dehydrogenase (EC 1.1.1.27); ATP phosphohydrolase (EC 3.8.1.3).

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Tonomura, 1967; Kazanawa *et al.*, 1971; Panet *et al.*, 1971; Vianna, 1975; Yates and Duance, 1978; Neet and Green, 1977; Möller *et al.*, 1980; Horgan, 1974; Dupont, 1977; Jorgensen *et al.*, 1978; Dean and Tanford, 1978). The downward deflection observed on these plots is typical of negative cooperativity (Levitsky, 1978).

Although it could be assumed, in principle, that this negative cooperativity could be attributed to substrate binding (Levitsky and Koshland, 1976), Reynolds *et al.* (1985) showed that both concepts are not always related. In fact, Meissner (1973) and Dupont (1977) obtained linear Scatchard plots for the binding of Mg^{2+}-ATP to the enzyme in the absence of free Ca^{2+}. On the other hand, even though Ca^{2+} may modulate the conformation of the (Ca^{2+}-Mg^{2+})-ATPase (Dupont, 1977; Dupont and Leigh, 1978; Inesi *et al.*, 1980; Ikemoto *et al.*, 1978; Nakamura *et al.*, 1979), Cable *et al.* (1985) concluded that Mg^{2+}-ATP binding does not show cooperativity in the presence of free Ca^{2+}.

Other interpretations have been suggested to explain the negative cooperativity observed in the ATPase activity, such as the existence of interaction between different sites in an oligomeric complex (Möller *et al.*, 1980) and the activation by an excess of Mg^{2+}-ATP through an effector site (Taylor and Hattan, 1979), although Cable *et al.* (1985) experimentally showed that this possibility is not likely.

An alternative intepretation was offered by Reynolds *et al.* (1985) based on the application of the principle of linked functions (Wyman, 1964) in which they proposed the existence of one binding site with two alternative conformational states, E_1 and E_2, which are able to bind Mg^{2+}-ATP. The observed cooperativity would then arise from this situation.

Cable *et al.* (1985) proposed that the negative cooperativity arises from the binding of Mg^{2+}-ATP to an intermediate species of the enzyme present during the catalytic cycle. This interpretation is supported by the observation that the Mg^{2+}-ATP binding to the phosphoenzyme complex accelerates the dephosphorylation step (Mitchinson *et al.*, 1982). Moreover, it has also been observed that Mg^{2+}-ATP reactivates the (Ca^{2+}-Mg^{2+})-ATPase activity previously inhibited by vanadate, suggesting that Mg^{2+}-ATP may bind to the enzyme–vanadate complex (Ortiz *et al.*, 1984) since this form is similar to the enzyme–phosphate complex. Therefore it is necessary to postulate only a single binding site for Mg^{2+}-ATP.

In the two former kinetic models (Reynolds *et al.*, 1985; Cable *et al.*, 1985) the corresponding steady-state rate equations are of the type 2:2 with respect to Mg^{2+}-ATP (a ratio of two quadratic polynomials). We show in this work that this type of equation does not explain the negative cooperativity with respect to Mg^{2+}-ATP in all ranges of concentration in which it is experimentally observed, i.e., from approximately 0.75 μM to 10 mM.

Gould *et al.* (1986) recently proposed a kinetic model for the (Ca^{2+}-Mg^{2+})-ATPase which, assuming a steady-state condition, would show a rate equation of the type 8:8 with respect to Mg^{2+}-ATP. This model considers two main enzymatic forms with different affinity for Mg^{2+}-ATP. Additionally, this model predicts that Mg^{2+}-ATP may bind to other intermediate enzymatic forms, with Mg^{2+}-ATP accelerating the slowest transition of the turnover.

In this work we suggest a model according to which Mg^{2+}-ATP may bind with different affinity to the three different enzymatic forms (E, E′, and *E) assumed to be present in the turnover of the (Ca^{2+}-Mg^{2+})-ATPase (Fernández-Belda *et al.*, 1984). The corresponding steady-state equation for the minimum model yields an equation of the type 3:3 for Mg^{2+}-ATP which satisfactorily explains the negative cooperativity for the hydrolysis of Mg^{2+}-ATP, at all the ranges of concentration in which this is observed.

Materials and Methods

Purification of the Enzyme

Sarcoplasmic reticulum vesicles were prepared from rabbit back and leg white muscles according to Nakamura *et al.* (1976), and the (Ca^{2+}-Mg^{2+})-ATPase was purified according to Method 2 of Meissner *et al.* (1973).

Analytical Assays

Protein concentration was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

ATPase activity was assayed at 25°C, using an Mg^{2+}-ATP regenerating system (Gómez-Fernández *et al.* 1980). The reaction mixture contained 100 mM triethanolamine, pH 7.0, 80 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA, 1 mM $CaCl_2$, 2 mM phosphoenolpyruvate, 0.24 mM NADH, 4 I.U./ml of pyruvate kinase, 6 I.U./ml of lactate dehydrogenase, and Mg^{2+}-ATP at different concentrations as indicated in each experiment. Protein concentration was 0.063 μM assuming a molecular weight of 115,000 for the ATPase (Warren *et al.*, 1974).

Results and Discussion

Mechanisms Which Yield 2:2 Type Rate Equations

If the rate equation under steady-state condition is deduced from some previous kinetic models proposed for the ATPase activity (Reynolds *et al.*,

1985; Cable *et al.*, 1985), the following is obtained:

$$\frac{v}{E_T} = \frac{\alpha_1|\text{ATP}| + \alpha_2|\text{ATP}|^2}{\beta_0 + \beta_1|\text{ATP}| + \beta_2|\text{ATP}|^2} \quad (1)$$

where α_1, α_2, β_0, β_1, and β_2 are dependent on the kinetic constants and Ca^{2+} concentration. Hence these models lead to equations of 2:2 type with respect to Mg^{2+}-ATP, and therefore the cooperativity will be found in a range of Mg^{2+}-ATP concentration where both binding affinities could participate.

There is agreement that the lowest dissociation constant of the enzyme for Mg^{2+}-ATP is 1 μM (Reynolds *et al.*, 1985; Cable *et al.*, 1985). However, discrepancies are found with respect to the higher dissociation constants. Cable *et al.* (1985) proposed 1 and 40 μM as values for the affinity constants, and consequently cooperativity will be found only in the low range of Mg^{2+}-ATP concentrations. On the other hand, Reynolds *et al.* (1985) proposed 1 and 800 μM, and therefore cooperativity could be found at high concentrations of Mg^{2+}-ATP.

It was also shown by Möller *et al.* (1980) using double reciprocal plots that cooperativity could be found in two different concentration ranges of Mg^{2+}-ATP, 0.75–50 μM and 0.1–10 mM. Although both authors used the experimental data of Möller *et al.* (1980), Cable *et al.* (1985) paid attention only to the low concentration values while Reynolds *et al.* (1985) plotted the data as activity versus log $|Mg^{2+}$-ATP| thus masking the cooperativity effects observed by Möller *et al.* (1980) at two different ranges of Mg^{2+}-ATP concentration. The existence of this double cooperativity renders inadequate the rate equations of 2:2 type mentioned above.

Mechanisms Which Yield n:n Type Rate Equation, where n ≥ 2

The model proposed by Gould *et al.* (1986), considered under the steady state, would yield a rate equation of the type 8:8 with respect to Mg^{2+}-ATP. These authors propose that the native enzyme can initially be found in two molecular states E_1 and E_2 showing different Mg^{2+}-ATP affinities and also that the substrate binds to the $E_1'PCa_2$ species accelerating the transition toward $E_2'PCa_2$. This model explains the negative cooperativity experimentally observed (Möller *et al.*, 1980) at high and low Mg^{2+}-ATP concentration ranges; however, in the mentioned model the transition ($E_2 \rightleftharpoons E_1$) cannot be reconciled with experimental data. Thus, the cooperative behavior of Ca^{2+} binding to (Ca^{2+}-Mg^{2+})-ATPase as measured by the intrinsic fluorescence technique (Fernández Belda *et al.*, 1984), as well as the measurements of Ca^{2+} binding kinetics (Dupont, 1982), can be fitted to a "sequential mechanism"

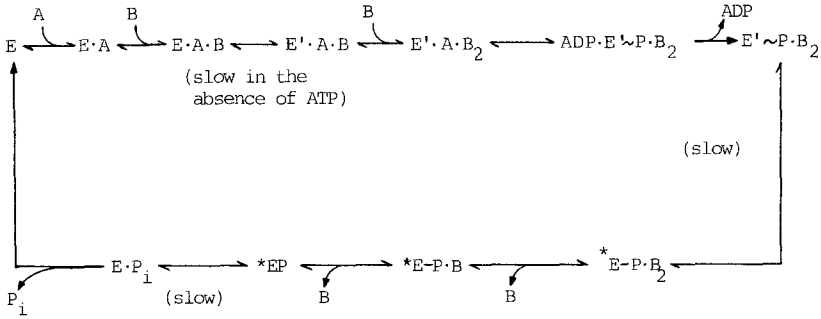

Fig. 1. Catalytic mechanism for the transport of Ca^{2+} and hydrolysis of Mg^{2+}-ATP by the (Ca^{2+}-Mg^{2+})-ATPase from sarcoplasmic reticulum. In this scheme (Inesi, 1985) A and B represent Mg^{2+}-ATP and Ca^{2+}, respectively. Note that the transition $A \cdot E \cdot B \rightleftharpoons A \cdot E' \cdot B$ is accelerated in the presence of ATP (Inesi, 1985).

(Hill and Inesi, 1982) as

$$E + Ca^{2+} \rightleftharpoons E \cdot Ca \rightleftharpoons E' \cdot Ca + Ca^{2+} \rightleftharpoons E' \cdot Ca_2$$

rather than to the model of Monod *et al.* (1965). Accordingly, we assume that the native enzyme resides in the E form and the presence of Ca^{2+} will give rise to the activated state $E' \cdot Ca_2$.

A New Proposed Mechanism Which Yields a 3 : 3 Type Rate Equation

The existence of this double cooperativity and the lack of a suitable model which could account for this fact prompted us to propose a more complete mechanism.

Our mechanism is based on the scheme proposed by other authors (Fernández-Belda *et al.* 1984; Inesi, 1985) in which three slow transitions corresponding to the interconversion of three different conformational states of the enzyme are supposed to take place during the catalytic cycle (Fig. 1). The native enzyme is in the state E and Mg^{2+}-ATP may bind to this enzymatic form in addition to $E' \sim P \cdot Ca_2$ and $*E - P \cdot Ca_2$, $*E - P \cdot Ca$, *EP, and $E \cdot P_i$ (generated in the turnover after ADP release) as indicated in Fig. 2.

Assuming values of 1, 20, and 500 μM for the dissociation constants of the three different enzymatic forms, i.e., E, *E, and E′ respectively, the accelerating mechanism induced by Mg^{2+}-ATP can be explained as follows. When Mg^{2+}-ATP binds to $E' \sim P \cdot Ca_2$ two slow steps can be bypassed, and the transition $E' \rightarrow E$ is accelerated (Fig. 2). The slow transition $E' \sim P \cdot Ca_2 \rightleftharpoons *E - PCa_2$ is accelerated by Mg^{2+}-ATP, producing $E' \sim P \cdot Ca_2 \cdot ATP \rightleftharpoons *E' - P \cdot Ca_2 \cdot ATP$. We introduce here the form *E′, since we consider that the transition in the presence of Mg^{2+}-ATP gives place to a form slightly different from *E. Nevertheless, if only the form *E, and not

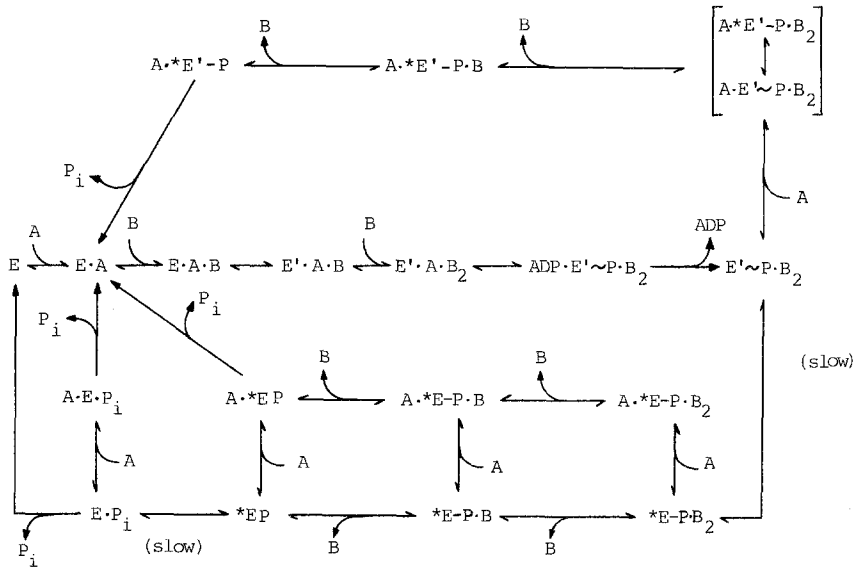

Fig. 2. Catalytic mechanism proposed for the (Ca^{2+}-Mg^{2+}) ATPase from sarcoplasmic reticulum. This mechanism includes all the possibilities of Mg^{2+}-ATP binding to all phosphorylated intermediates.

*E′, is considered as done by Gould *et al.* (1986), the mechanism remains totally valid. However, when Mg^{2+}-ATP binds to the phosphoenzyme with low phosphorylation potential (*E − P · Ca_2, *E − P · Ca, and *EP), one slow step will be bypassed and the transition *E → E will be accelerated. Thus, the activation at low Mg^{2+}-ATP concentrations will be explained by the binding of Mg^{2+}-ATP to the enzymatic forms appearing after the slow transition E′ ~ P · Ca_2 ⇌ *E − P · Ca_2 with a dissociation constant of about 20 μM; however, the activation effect at high concentrations of Mg^{2+}-ATP can be explained by the binding of Mg^{2+}-ATP to E′ ~ P · Ca_2 with a lower affinity (K_9 = 500 μM).

The deduction of the rate equation for the mechanism written in Fig. 2 is a rather complex matter (6 : 6 with respect to Mg^{2+}-ATP), and in order to explain the experimental results the development of a minimum mechanism of the type shown in Fig. 3A is sufficient. In this simplified scheme the slow steps which will be accelerated by Mg^{2+}-ATP are kept as essentials. The Mg^{2+}-ATP binding steps are supposed to be in rapid equilibrium.

The rate equation obtained in this case and with factorizing (see Appendix) according to Fig. 3B is of a 3 : 3 type,

$$\frac{v}{E_T} = \frac{a_1|ATP| + a_2|ATP|^2 + a_3|ATP|^3}{b_0 + b_1|ATP| + b_2|ATP|^2 + b_3|ATP|^3} \qquad (2)$$

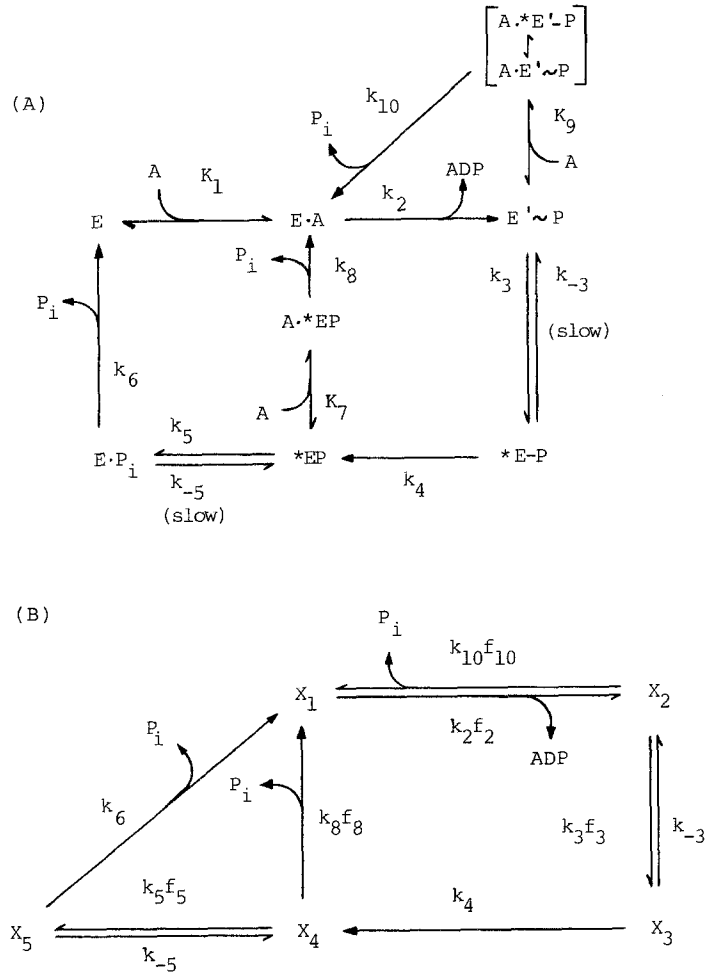

Fig. 3. (A) Minimum catalytic mechanism proposed here for the (Ca^{2+}-Mg^{2+})-ATPase from sarcoplasmic reticulum. Mechanism derived from the one shown in Fig. 2 but including only the Mg^{2+}-ATP effect and binding to the *EP and E′ ~ P intermediates. Note that Ca^{2+} has been omitted in this scheme for simplicity. (B) Factorizing scheme used to analyze the catalytic mechanism proposed in (A), to obtain the corresponding rate equation. The concentration factors are included in this scheme and are defined in the Appendix section. The meaning of the X_i (i = 1–5) enzymatic species is evident by comparing (A) and (B).

The coefficients of this equation are defined in the Appendix. This equation satisfactorily explains the experimental results as discussed below.

Our experimental results of v versus Mg^{2+}-ATP concentrations closely agree with those shown by Möller *et al.* (1980). They show cooperativity

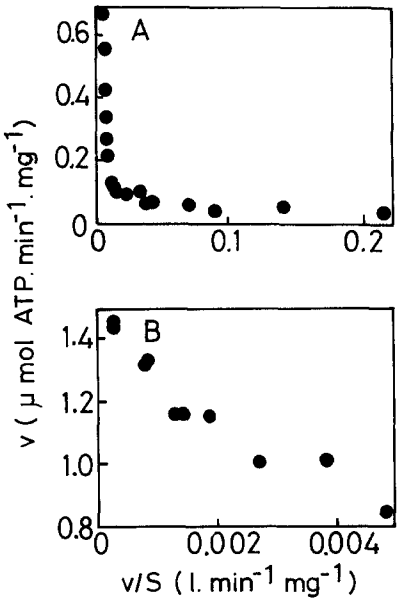

Fig. 4. Variation of ATPase activity by changing Mg^{2+}-ATP concentration. Experimental data obtained with purified (Ca^{2+}-Mg^{2+})-ATPase from sarcoplasmic reticulum for the variation of ATPase activity (μmol ATP min^{-1} mg^{-1}) when Mg^{2+}-ATP concentration was changed from 1 to 120 μM in (A) and from 0.17 to 5 mM in (B). Eadie–Hofstee plots of the experimental data are shown.

when plotting v versus v/S (and also $1/v$ vs. $1/S$, data not shown) at both low (Fig. 4A) and high (Fig. 4B) concentrations of Mg^{2+}-ATP.

Computer simulation of Eq. (2) was performed using, for the constants, the values shown in Table I. Cooperativity is observed at both low and high concentrations of Mg^{2+}-ATP (Figs. 5A and 5B, respectively), in agreement with our experimental results and also with those of Möller *et al.* (1980).

Table I. Rate Constant and Dissociation Constant Values[a]

Rate constant (s^{-1})	Dissociation constant (M)
$k_2 = 40$ $k_{-5} = 60^b$	$K_1 = 1 \times 10^{-6b}$
$k_3 = 48^b$ $k_6 = 600^b$	$K_7 = 2 \times 10^{-5}$
$k_{-3} = 35^b$ $k_8 = 200$	$K_9 = 5 \times 10^{-4}$
$k_4 = 70$ $k_{10} = 250$	
$k_5 = 60^b$	

[a]These constants were used for the simulation of the kinetic behavior of the mechanism shown in Fig. 3. Some values represent experimental estimates, but others were arbitrarily chosen in order to reproduce the cooperative behavior.
[b]From Fernández-Belda *et al.* (1984).

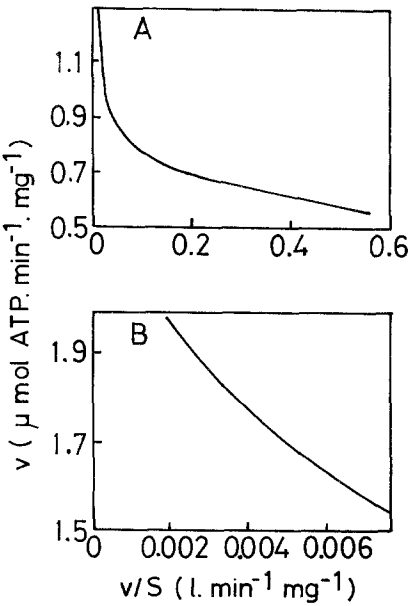

Fig. 5. Simulated steady-state rate values calculated by using Eq. (2). Eadie–Hofstee plots of theoretical data are shown. The values of the constants used here are given in Table I. Mg^{2+}-ATP concentration was changed from 1 to 100 μM in (A) and from 0.2 to 1 mM in (B).

It should be noted that our mechanism has been simplified, and hence cannot be fitted to the rates of the experimental results; however, it satisfactorily describes the effect of Mg^{2+}-ATP and the cooperative behavior.

If Eq. (2) is reduced to a 2:2 type for Mg^{2+}-ATP by raising to infinity the binding of Mg^{2+}-ATP to any of the E′ or *E forms, eqs. (3) and (4) are obtained (see the Appendix for the definition of the coefficients):

$$\frac{v}{E_T} = \frac{a'_1|\mathrm{ATP}| + a'_2|\mathrm{ATP}|^2}{b'_0 + b'_1|\mathrm{ATP}| + b'_2|\mathrm{ATP}|^2} \tag{3}$$

$$\frac{v}{E_T} = \frac{a''_1|\mathrm{ATP}| + a''_2|\mathrm{ATP}|^2}{b''_0 + b''_1|\mathrm{ATP}| + b''_2|\mathrm{ATP}|^2} \tag{4}$$

It is important to point out that these equations cannot explain at the same time the cooperativity effects at both low (Figs. 6A and 7A) and high concentrations of Mg^{2+}-ATP (Figs. 6B and 7B). On the other hand, if our results are plotted as v versus log |Mg^{2+}-ATP|, then cooperativity at low and high Mg^{2+}-ATP concentrations cannot be observed (data not shown), thus explaining why Reynolds *et al.* (1985), who used this type of plot, found apparently satisfactory a 2:2 type of equation for the experimental results

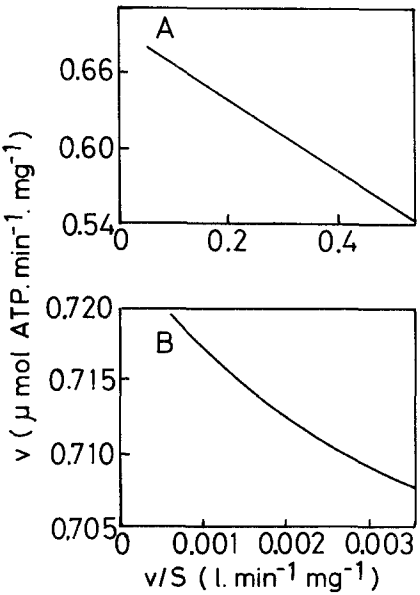

Fig. 6. Simulated steady-state rate values calculated by using Eq. (3). Eadie–Hofstee plots of the theoretical data are shown. The values of the constants used here are given in Table I. Mg^{2+}-ATP concentration was changed from 1 to 100 μM in (A) and from 0.2 to 1 mM in (B).

shown by Moller *et al.* (1980). Something similar happens with the results of Gould *et al.* (1986), since although this mechanism might explain the negative cooperativity in the low and high Mg^{2+}-ATP levels, the results obtained at low concentrations of ATP are masked by the use of the plot of v versus log $|Mg^{2+}\text{-ATP}|$.

Concluding Remarks

It should be remarked that the acceleration of the release of P_i by the addition of Mg^{2+}-ATP has been experimentally shown (de Meis and de Mello, 1973; McIntosh and Boyer, 1983). However, it is not possible to calculate, from these experiments, the dissociation constants for Mg^{2+}-ATP in the species $E' \sim P \cdot Ca_2$, $*E - P \cdot Ca$, and *EP, since the first evolves toward the second, the second toward the third, and all of them release P_i (see Fig. 2). The formation of $E \cdot P_i$ from E and P_i enables one to study the liberation of P_i from such a complex in an independent way (McIntosh and Boyer, 1983). Studies of the decomposition of the *E-vanadate complex also

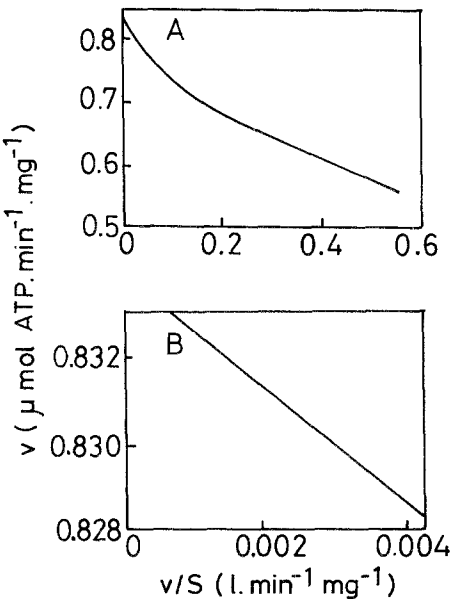

Fig. 7. Simulated steady-state rate values calculated by using Eq. (4). Eadie–Hofstee plots of the theoretical data are shown. The values of the constants used here are given in Table I. Mg^{2+}-ATP concentration was changed from 1 to 100 μM in (A) and from 0.2 to 1 mM in (B).

gave information related to the release of P_i from *EP, assuming a structural analogy between *EP and *E-vanadate (Ortiz *et al.*, 1984).

A mechanism similar to that proposed by Petterson (1986) for hexokinase type L_I from wheat germ is proposed from the (Ca^{2+}-Mg^{2+})-ATPase from sarcoplasmic reticulum. In that case glucose activates the enzyme upon binding to an enzyme-product complex (E-ADP) which is structurally analogous to the enzyme–substrate complex (E-ATP). In our case, the substrate Mg^{2+}-ATP should be capable of binding to the enzyme species E′ ~ P and *EP (Fig. 3). The binding of Mg^{2+}-ATP to these species is possible not because of the structural analogy in the case of hexokinase but because of the small size of P_i which allows accessibility of Mg^{2+}-ATP to the binding site. Note that a single binding site for Mg^{2+}-ATP is postulated in the enzymatic forms E, E′, and *E.

In conclusion, we propose a kinetic mechanism for the hydrolysis of Mg^{2+}-ATP by the (Ca^{2+}-Mg^{2+})-ATPase from sarcoplasmic reticulum, which explains the negative cooperativity observed at both high and low Mg^{2+}-ATP concentrations, and also other experimental observations such as the acceleration of P_i release by Mg^{2+}-ATP (de Meis and de Mello, 1973; McIntosh and Boyer, 1983) and the existence of three different and kinetically

related E, E′, and *E enzyme forms, (Fernández-Belda *et al.*, 1984; Inesi, 1985). The simplest equation for the proposed mechanism must be of 3:3 type for Mg^{2+}-ATP in order to obtain agreement between experimental and simulated data.

Appendix

Deduction of the Rate Equations

The rate equations are obtained by analyzing the kinetic mechanism shown in Fig. 3A, factorizing according to the scheme of Fig. 3B. The concentration factors are defined as follows

$$f_2 = |\mathrm{ATP}|/(K_1 + |\mathrm{ATP}|)$$
$$f_3 = K_9/(K_9 + |\mathrm{ATP}|)$$
$$f_5 = K_7/(K_7 + |\mathrm{ATP}|)$$
$$f_8 = |\mathrm{ATP}|/(K_7 + |\mathrm{ATP}|)$$
$$f_{10} = |\mathrm{ATP}|/(K_9 + |\mathrm{ATP}|)$$

Equation of Type 3:3

This corresponds to Eq. (2) described in the Results and Discussion section. The coefficients are defined as follows:

$$a_1 = k_2k_3k_4k_5k_6K_7K_9$$

$$a_2 = (k_2k_3k_4k_{-5}k_8 + k_2k_3k_4k_6k_8)K_9 + (k_2k_{-3}k_{-5}k_8k_{10} + k_2k_{-3}k_5k_6k_{10})K_7$$

$$a_3 = k_2k_4k_{10}k_{-5}k_8 + k_2k_{-3}k_{10}k_{-5}k_8 + k_2k_4k_{10}k_6k_8 + k_2k_{-3}k_{10}k_6k_8$$

$$b_0 = k_3k_4k_5k_6K_1K_7K_9$$

$$b_1 = (k_3k_4k_{-5}k_8 + k_3k_4k_6k_8)K_1K_9 + (k_4k_5k_6k_{10} + k_{-3}k_5k_6k_{10})K_1K_7 + (k_3k_4k_5k_6 + k_2k_3k_4k_{-5} + k_2k_3k_4k_6 + k_2k_3k_4k_5 + k_2k_3k_5k_6 + k_2k_4k_5k_6 + k_2k_{-3}k_5k_6)K_7K_9$$

$$b_2 = (k_4k_{10}k_{-5}k_8 + k_{-3}k_{10}k_{-5}k_8 + k_4k_{10}k_6k_8 + k_{-3}k_{10}k_6k_8)K_1 + (k_4k_5k_6k_{10} + k_{-3}k_{10}k_5k_6 + k_2k_4k_5k_6 + k_2k_{-3}k_5k_6)K_7 + (k_3k_4k_{-5}k_8 + k_3k_4k_6k_8 + k_2k_3k_4k_{-5} + k_2k_3k_4k_6 + k_2k_3k_{-5}k_8 + k_2k_3k_6k_8 + k_2k_4k_{-5}k_8 + k_2k_4k_6k_8 + k_2k_{-3}k_{-5}k_8 + k_2k_{-3}k_6k_8)K_9$$

$$b_3 = k_4k_{10}k_{-5}k_8 + k_{-3}k_{-5}k_8k_{10} + k_4k_{10}k_6k_8 + k_{-3}k_6k_{10}k_8 + k_2k_4k_{-5}k_8 + k_2k_4k_6k_8 + k_2k_{-3}k_{-5}k_8 + k_2k_{-3}k_6k_8$$

Note that some of these constants are apparent and depend on the Ca^{2+} concentration which was omitted when Fig. 2 was simplified to Fig. 3A. In this work the Ca^{2+} concentration is kept constant and only the effect of changing Mg^{2+}-ATP concentration is studied.

Equations of type 2 : 2

Equation (3) of Results and Discussion Section. This equation is obtained from Eq. (2), setting $K_7 = \infty$ and $k_8 = 0$ so that the binding of Mg^{2+}-ATP to *EP is not allowed and Mg^{2+}-ATP will bind to E′ ~ P (Fig. 3). The coefficients are defined as

$$a_1' = k_2k_3k_4k_5k_6K_9$$

$$a_2' = k_2k_{-3}k_{10}k_5k_6$$

$$b_0' = k_3k_4k_5k_6K_1K_9$$

$$b_1' = (k_4k_5k_6k_{10} + k_{-3}k_5k_6k_{10})K_1 + (k_3k_4k_5k_6 + k_2k_3k_4k_{-5} + k_2k_3k_4k_6 + k_2k_3k_4k_5 + k_2k_3k_5k_6 + k_2k_4k_5k_6 + k_2k_{-3}k_5k_6)K_9$$

$$b_2' = k_4k_5k_6k_{10} + k_{-3}k_{10}k_5k_6 + k_2k_4k_5k_6 + k_2k_{-3}k_5k_6$$

Equation (4) of Results and Discussion Section. This equation is obtained from Eq. (2), setting $K_9 = \infty$ and $k_{10} = 0$ so that the binding of Mg^{2+}-ATP to E′ ~ P is not allowed and Mg^{2+}-ATP will bind to *EP (Fig. 3). The coefficients are defined as

$$a_1'' = k_2k_3k_4k_5k_6K_7$$

$$a_2'' = k_2k_3k_4k_{-5}k_8 + k_2k_3k_4k_6k_8$$

$$b_0'' = k_3k_4k_5k_6K_1K_7$$

$$b_1'' = (k_3k_4k_{-5}k_8 + k_3k_4k_6k_8)K_1 + (k_3k_4k_5k_6 + k_2k_3k_4k_{-5} + k_2k_3k_4k_6 + k_2k_3k_4k_5 + k_2k_3k_5k_6 + k_2k_4k_5k_6 + k_2k_{-3}k_5k_6)K_7$$

$$b_2'' = k_3k_4k_{-5}k_8 + k_3k_4k_6k_8 + k_2k_3k_4k_{-5} + k_2k_3k_4k_6 + k_2k_3k_{-5}k_8 + k_2k_3k_6k_5 + k_2k_4k_{-5}k_8 + k_2k_4k_6k_8 + k_2k_{-3}k_{-5}k_8 + k_2k_{-3}k_6k_8$$

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References

Cable, M. B., Fherer, J. J., and Briggs, F. N. (1985). *Biochemistry* **24**, 5612–5619.
Dean, W. L., and Tanford, C. (1978). *Biochemistry* **17**, 1683–1690.
de Meis, L., and de Mello, M. C. F. (1973). *J. Biol. Chem.* **248**, 3691–3701.
Dupont, Y. (1977). *Eur. J. Biochem.* **72**, 185–190.
Dupont, Y. (1982). *Biochim. Biophys. Acta* **688**, 75–87.
Dupont, Y., and Leigh, J. B. (1978). *Nature* (*London*) **273**, 396–398.
Fernández-Belda, F., Kurzmack, M., and Inesi, G. (1984). *J. Biol. Chem.* **259**, 9687–9698.
Gómez-Fernández, J. C., Goñi, F. M., Bach, D., Restall, C. J., and Chapman, D. (1980). *Biochim. Biophys. Acta* **598**, 502–516.
Gould, G. W., East, J. M., Froud, R. J., McWhirter, J. M., Stefanova, H. I., and Lee, A. G. (1986). *Biochem. J.* **237**, 217–227.
Hill, T., and Inesi, G. (1982). *Proc. Natl. Acad. Sci. USA* **79**, 3978–3982.
Horgan, D. J. (1974). *Arch. Biochem. Biophys.* **162**, 6–11.
Ikemoto, N., Morgan, J. F., and Yamada, S. (1978). *J. Biol. Chem.* **253**, 8027–8033.
Inesi, G. (1985). *Annu. Rev. Physiol.* **47**, 573–601.
Inesi, G., Kuezmack, M., Coan, C., and Lewis, D. E. (1980). *J. Biol. Chem.* **255**, 3025–3031.
Jorgensen, K. E., Lind, K. E.., Roigaard-Petersen, H., and Möller, J. V. (1978). *Biochem. J.* **169**, 489–498.
Kanazawa, T., Yamada, S., Yamamoto, T., and Tonomura, Y. (1971). *J. Biochem. (Tokyo)* **70**, 95–123.
Levitsky, A. (1978). *Quantitative Aspects of Allosteric Mechanism*, Springer-Verlag, Berlin.
Levitsky, A., and Koshland, D. E., Jr. (1976). *Curr. Top. Cell Regul.* **10**, 1–40.
Lowry, O. H., Rosebrough, N. J., Farr, A. C., and Randall, R. J. (1951). *J. Biol. Chem.* **193**, 265–275.
McIntosh, D. B., and Boyer, P. D. (1983). *Biochemistry* **22**, 2867–2875.
Meissner, G. (1973). *Biochim. Biophys. Acta* **298**, 906–926.
Meissner, G., Conner, G., and Fleischer, S. (1973). *Biochim. Biophys. Acta* **298**, 246–269.
Mitchinson, C., Wilderspin, A. F., Trinnaman, B. J., and Green, N. M. (1982). *FEBS Lett.* **146**, 87–92.
Möller, J. V., Lind, K. E., and Andersen, J. P. (1980). *J. Biol. Chem.* **255**, 1912–1920.
Monod. J., Wyman, J., and Changeux, J. (1965). *J. Mol. Biol.* **12**, 88–118.
Nakamura, H., Jilka, R. L., Boland, R., and Martonosi, A. N. (1976). *J. Biol. Chem.* **251**. 5414–5423.
Nakamura, Y., Tonomura, Y., and Hagiwara, B. (1979). *J. Biochem.* (*Tokyo*) **86**, 443–446.
Neet, K. E., and Green, N. M. (1977). *Arch. Biochem. Biophys.* **178**, 588–597.
Ortiz, A., García-Carmona, F., García-Cánovas, F., and Gómez-Fernández, J. C. (1984). *Biochem. J.* **221**, 213–222.
Panet, R., Pick, U., and Selinger, Z. (1971). *J. Biol. Chem.* **246**, 7349–7356.
Petterson, G. (1986). *Eur. J. Biochem.* **154**, 167–170.
Reynolds, J. A., Johnson, E. A., and Tanford, C. (1985). *Proc. Natl. Acad. Sci. USA* **82**, 3658–3661.
Taylor, J. S., and Hattan, D. (1979). *J. Biol. Chem.* **254**, 4402–4407.
Vianna, A. L. (1975). *Biochim. Biophys. Acta* **410**, 389–406.
Warren, G. B., Toon, P. A., Birdsall, N. J. M., Lee, A. G., and Metcalfe, J. C. (1974). *Proc. Natl. Acad. Sci. USA* **71**, 622–626.
Wyman, J. (1964). *Adv. Protein Chem.* **19**, 223–286.
Yamamoto, T., and Tonomura, Y. (1967). *J. Biochem.* (*Tokyo*) **62**, 558–575.
Yates, D. W., and Duance, V. C. (1978). *Biochem. J.* **159**, 719–728.