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THE EFFECT OF THE Ca^{2+}/Mg^{2+} CONCENTRATION RATIO ON PLACENTAL (Ca^{2+}–Mg^{2+})-ATPase ACTIVITY.

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SUMMARY

The velocity of the reaction of a (Ca^{2+}–Mg^{2+})stimulated ATPase in placental plasma membranes of the guinea pig was found to depend on the $[Mg^{2+}]/[Ca^{2+}]$ concentration ratio. The observed activation curve of the enzyme while changing the concentration ratio agrees with the theoretical equation, derived for the case of two ions activating the same site while the enzyme is saturated. Because of the good agreement, we conclude that the two divalent cations, Ca^{2+} and Mg^{2+}, activate this placental ATPase at the same site. The equation may be used for other enzymes to determine whether or not two ions activate the same site, and to predict the velocity of the reaction for a given ion concentration ratio.

INTRODUCTION

A Ca^{2+} and/or Mg^{2+}-stimulated ATPase is found in several tissues[1–5]. Guinea pig placental plasma membranes were found to contain such a Ca^{2+}- or Mg^{2+}-stimulated ATPase[6]. This enzyme does not require Na^+ or K^+ for activation and is not inhibited by ouabain, but can be activated by either Ca^{2+} or Mg^{2+} in the absence of the other. However, the requirements for Ca^{2+} and Mg^{2+} differ for each ATPase. The present paper offers a method for determining whether the two ions activate the same site. From the kinetic behaviour of the enzyme in the presence of the two ions, we suggest that the two ions, Ca^{2+} and Mg^{2+}, activate the same site on the enzyme. To prove this postulate an equation has been derived based on the assumptions that the two ions each form a complex with ATP which serves as the substrate for the enzyme on the same site. Additional assumptions are that the association of Ca^{2+}, Mg^{2+} and ATP directly with the enzyme is very low compared with the Ca–ATP and Mg–ATP association constants.

We assume that Ca^{2+} reacts in the following way:

$$Ca^{2+} + ATP \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Ca\text{-}ATP \qquad (1)$$

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The stability constant of the Ca–ATP complex will be:

$$K_1 = \frac{k_1}{k_2} = \frac{[\text{Ca–ATP}]}{[\text{Ca}^{2+}]\,[\text{ATP}]} \tag{2}$$

$$\text{Ca–ATP} + E \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E\text{–Ca–ATP} \tag{3}$$

$$K_2 = \frac{k_4}{k_3} = \frac{[\text{Ca–ATP}]\,[E]}{[E\text{–Ca–ATP}]} \tag{4}$$

$$E\text{–Ca–ATP} \xrightarrow{k_5} E + \text{Products} \tag{5}$$

$$v_1 = k_5[E\text{–Ca–ATP}] \tag{6}$$

Similarly for Mg^{2+} the following holds:

$$\text{Mg}^{2+} + \text{ATP} \underset{k_7}{\overset{k_6}{\rightleftharpoons}} \text{Mg–ATP} \tag{7}$$

The stability constant of Mg–ATP complex will be:

$$K_3 = \frac{k_6}{k_7} = \frac{[\text{Mg–ATP}]}{[\text{Mg}^{2+}]\,[\text{ATP}]} \tag{8}$$

$$E + \text{Mg–ATP} \underset{k_9}{\overset{k_8}{\rightleftharpoons}} E\text{–Mg–ATP} \tag{9}$$

$$K_4 = \frac{k_9}{k_8} = \frac{[\text{Mg–ATP}]\,[E]}{[E\text{–Mg–ATP}]} \tag{10}$$

$$E\text{–Mg–ATP} \xrightarrow{k_{10}} E + \text{Products} \tag{11}$$

$$v_2 = k_{10}\,[E\text{–Mg–ATP}] \tag{12}$$

Since there is competition between Ca^{2+} and Mg^{2+} for ATP in the first stage and again between Ca–ATP and Mg–ATP for the enzyme (*E*), the ratio [*E*–Ca–ATP] to [*E*–Mg–ATP] after introducing the terms obtained from Eqns. 2, 4, 8 and 10, will be:

$$\frac{[E\text{–Ca–ATP}]}{[E\text{–Mg–ATP}]} = \frac{K_1K_4\,[\text{Ca}^{2+}]}{K_3K_2\,[\text{Mg}^{2+}]} \tag{13}$$

Saturating the enzyme by using at least one of the ions at the optimal concentration, the total (*E*) will form complexes with both substrates in the following way:

$$\frac{[E\text{–Mg–ATP}]}{[E]} = \frac{100}{\dfrac{K_1K_4[\mathrm{Ca}^{2+}]}{K_3K_2[\mathrm{Mg}^{2+}]} + 1} \tag{14}$$

This is the fraction of the enzyme which is complexed with Mg–ATP. Since the enzyme is saturated, the rest of the enzyme which is not complexed with Mg–ATP, will complex with Ca–ATP. Thus *E*–Ca–ATP complex in percent of total enzyme will be:

$$\frac{[E\text{–Ca–ATP}]}{[E]} = 100 - \frac{100}{\dfrac{K_1K_4[\mathrm{Ca}^{2+}]}{K_3K_2[\mathrm{Mg}^{2+}]} + 1} \tag{15}$$

Since the total activity is

$$A = v_1 + v_2 = k_5\,[\text{E–Ca–ATP}] + k_{10}\,[\text{E–Mg–ATP}] \tag{16}$$

we can write:

$$A = \left(\frac{100}{\dfrac{K_1K_4[\mathrm{Ca}^{2+}]}{K_3K_2[\mathrm{Mg}^{2+}]}} + 1\right)k_{10} + \left(100 - \frac{100}{\dfrac{K_1K_4[\mathrm{Ca}^{2+}]}{K_3K_2[\mathrm{Mg}^{2+}]} + 1}\right)k_5 \tag{17}$$

Introducing the constants into Eqn. 17 and substituting V_1 for k_5, V_2 for k_{10} we get the total activity in percent by referring to the V_1 for Ca^{2+} as 1.0.

$$A = \left(\frac{100}{\dfrac{K_1K_4[\mathrm{Ca}^{2+}]}{K_3K_2[\mathrm{Mg}^{2+}]} + 1}\right)\mathrm{V}_2 + \left(100 - \frac{100}{\dfrac{K_1K_4[\mathrm{Ca}^{2+}]}{K_3K_2[\mathrm{Mg}^{2+}]} + 1}\right)V_1 \tag{18}$$

Since we assume that the last stages of the pathway for Eqns. 5 and 11 are irreversible we can use $(K_m)_1$ instead of K_2 and $(K_m)_2$ instead of K_4. (K_2 and K_4 are the dissociation constants). K_1 and K_3 are the stability constants of Ca–ATP and Mg–ATP. Thus the only variable is either [Ca²⁺] or [Mg²⁺] while the other ion is at optimal concentration.

Finally the formula will be:

$$A = \left(\frac{100}{\dfrac{K_1(K_m)_2[\mathrm{Ca}^{2+}]}{K_3(K_m)_1[\mathrm{Mg}^{2+}]} + 1}\right)V_2 + \left(100 - \frac{100}{\dfrac{K_1(K_m)_2[\mathrm{Ca}^{2+}]}{K_3(K_m)_1[\mathrm{Mg}^{2+}]} + 1}\right)V_1 \tag{19}$$

MATERIALS AND METHODS

Guinea pig placental plasma membranes were isolated according to the method of Post and Sen[7]. The experiment was carried out as described in previous work[6]. The amount of inorganic phosphate released was measured by the Gomori[8] method and the protein in the sample by the procedure of Lowry *et al.*[9].

RESULTS

The activation curves obtained by either of the two ions, Ca^{2+} and Mg^{2+}, are rectangular hyperbolas of typical substrate concentration curves (Fig. 1).

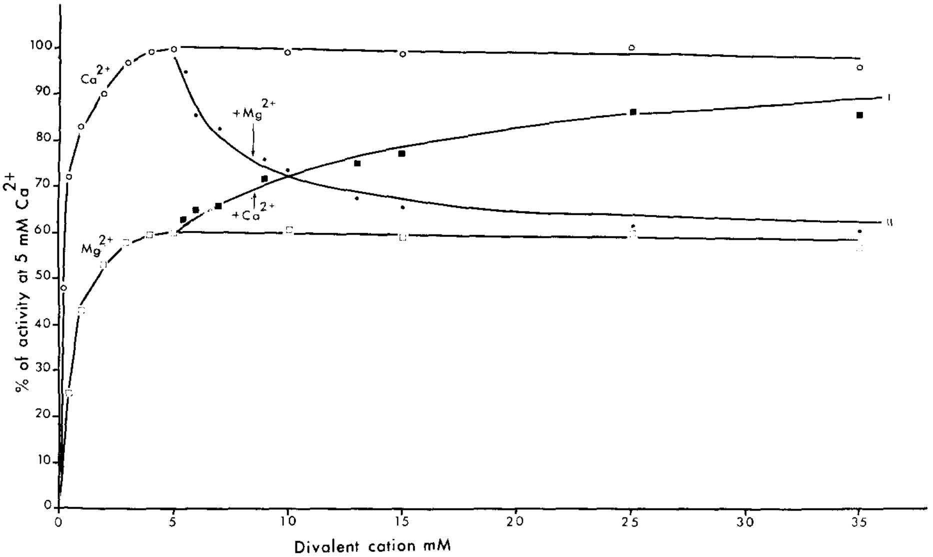

Fig. 1. The effects of Ca^{2+} and Mg^{2+} on ATP hydrolysis. The incubation medium contained 70 mM NaCl, 20 mM Tris–HCl (pH 8.2), 5 mM Na_2ATP and Ca^{2+} or Mg^{2+} or Ca^{2+} + Mg^{2+} in concentration as indicated. Results expressed as percent of activity at 5 mM Ca^{2+} which ranged between 15 and 22 µmole/P_i per mg protein in 30 min at 37°. ○—○ activation by Ca^{2+}, □—□ activation by Mg^{2+}; I, II, theoretical curves obtained by using the equation. ○, ●, experimental values for 5 mM Ca^{2+} and increasing Mg^{2+} concentrations; □, ■, experimental values for 5 mM Mg^{2+} and increasing Ca^{2+} concentrations.

Adding increasing amounts of Mg^{2+} to 5 mM Ca^{2+} we obtained inhibition of ATP hydrolysis and consequently stimulation by adding increasing amounts of Ca^{2+} to 5 mM Mg^{2+}. The results obtained are shown in Fig. 1. The apparent K_m (calculated from a Lineweaver-Burk plot) was 0.24 mM for Ca^{2+} and 0.52 mM for Mg^{2+}, for Ca^{2+} the $V_1 = 1$, and for Mg^{2+}, $V_2 = 0.6$. The stability constants for Ca–ATP and Mg–ATP[10] (K_1 and K_3) are $\log K_1 = 3.9$ and $\log K_3 = 4.5$. Curves I and II in Fig. 1

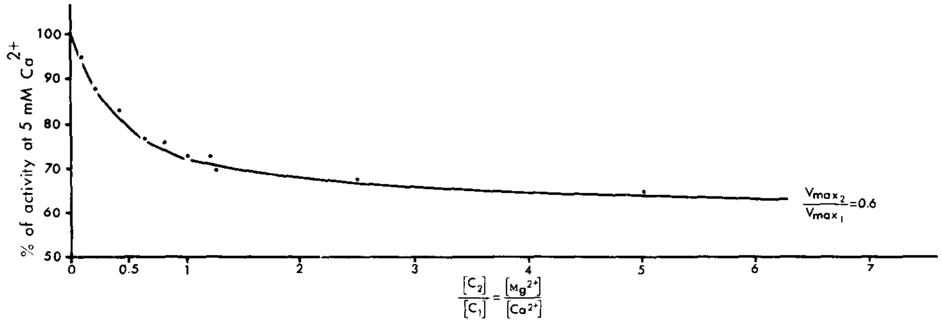

Fig. 2. The effect of changing the concentration ratio of Mg^{2+} to Ca^{2+}, while at least one cation is at the optimal concentration of 5 mM. ———, theoretical, calculated curve; ●, experimental values obtained when the V_2 for Mg^{2+} was 60% of V_1 for Ca^{2+}.

are the theoretical curves obtained by using Eqn. 19. Each experimental value was the mean of samples done in triplicate and the whole experiment repeated four times. The goodness of fit between the theoretical curves and the experimental points was tested by using the χ^2 test, and the values were for each of four experiments: $0.99 > P_1 > 0.97$; $0.995 > P_2 > 0.99$; $P_3 = 0.97$; $0.9 > P_4 > 0.75$.

Since the concentration ratio is the only variable and all other values are constant, it is possible to plot A (activity) against $[Mg^{2+}]/[Ca^{2+}]$ while either Mg^{2+} or Ca^{2+} is at optimal concentration (5 mM). The calculated theoretical curve so obtained by using the above equation and the experimental results are shown in Fig. 2.

DISCUSSION

The formula presented in the paper will enable us to predict the activity which will be obtained by using ions in any concentration ratio while the enzyme is saturated. If two ions behave according to this equation we may conclude that they activate the same site on the enzyme. Though it is possible to adopt this equation to predict the activity of an unsaturated enzyme, the prediction will be less accurate than for the saturated enzyme.

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REFERENCES

1 O. L. Martin, M. J. Melancon and H. F. Deluca, *Biochem. Biophys. Res. Commun.*, 35 (1969) 819.
2 D. K. Parkinson and I. C. Radde., *Biochim. Biophys. Acta*, 242 (1971) 238.
3 D. H. MacLennan, *J. Biol. Chem.*, 245 (1970) 4508.
4 S. Berl and S. Puszkin, *Biochemistry*, 9 (1970) 2048.
5 H. J. Schatzmann and F. F. Vincenzi, *J. Physiol.*, 201 (1969) 369.
6 Y. Shami and I. C. Radde, *Biochim. Biophys. Acta*, 249 (1971) 345.
7 R. L. Post and A. K. Sen, *Methods Enzymol.*, 10 (1967) 762.
8 G. Gomori, *J. Lab. Clin. Med.*, 27 (1942) 955.
9 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 192 (1951) 265.
10 M. E. Hyde and L. Rimai, *Biochemistry*, 10 (1971) 1121.