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The Thermodynamic Efficiency of the Ca^{2+}–Mg^{2+}-ATPase Is One Hundred Percent[1]

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Abstract

The thermodynamic efficiency of the Ca^{2+}–Mg^{2+}-ATPase of skeletal sarcoplasmic reticulum has been evaluated by comparing the Ca^{2+} gradient established with the ATP/(ADP*P_i) ratio. The evaluation was made at an external Ca^{2+} level (4.7×10^{-8} M) which is below the K_m value of 7×10^{-8} M. The Mg–ATP and phosphate concentrations were held constant (0.1 mM) and the ADP concentration was varied. Maximal uptake to an internal free Ca^{2+} concentration of 17 mM was observed at infinite ATP/(ADP*P_i) ratio (absence of ADP). This corresponds to a $[Ca^{2+}]_i/[Ca^{2+}]_0$ gradient of 3.6×10^5. A Ca^{2+} gradient one-half as large was observed at an ATP/(ADP*P_i) ratio of 3.5×10^3 M^{-1}. The square of the Ca^{2+} gradient is shown to be proportional to the ATP/(ADP*P_i) ratio, for finite values of the latter. The proportionality constant is identical to the equilibrium constant for hydrolysis of ATP (9.02×10^6 M) under these conditions (0.1 mM Mg^{2+}, 30°C). The intrinsic thermodynamic efficiency of the pump is shown to be 100%, with a maximal uncertainty of 3%. The efficiency is lower under less optimal conditions, when the pump is inhibited and passive leak processes compete.

Key Words: Ca^{2+} transport; sarcoplasmic reticulum; Ca^{2+}–Mg^{2+}-ATPase; ion transport; phosphate potential; bioenergetics; ion gradient; 1-anilino-8-naphthalene sulfonate.

Introduction

Calcium uptake by the sarcoplasmic reticulum (SR)[4] of the skeletal muscle (Hasselbach and Makinose, 1963) is driven by a Ca^{2+}-dependent ATPase

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[4]Abbreviations: SR, sarcoplasmic reticulum; ANS, 1-anilino-8-naphthalenesulfonate; $[Ca^{2+}]_i$, free internal Ca^{2+} concentration in the SR lumen; Tris, tris(hydroxyl)aminomethane; Hepes, 4-(2-hydroxyl)-1-piperazineethanesulfonic acid; P_i, inorganic phosphate.

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pump. Its enzymatic mechanism has been delineated in a large number of studies (MacLennan and Holland, 1975; Weber *et al.*, 1966; Kanazawa *et al.*, 1971; Inesi, 1972; Froehlich and Taylor, 1975, 1976; Hasselbach, 1978; Tada *et al.*, 1978; DeMeis and Vianna, 1979; Chiu and Haynes, 1980; Haynes and Mandveno, 1983). The present study is concerned with the tightness of coupling between ATP hydrolysis and Ca^{2+} transport. The present communication shows that the intrinsic thermodynamic efficiency of the pump is 100%.

We have previously studied the effect of the ATP/(ADP*P_i) potential on the free internal Ca^{2+} concentration in the SR lumen (Dixon *et al.*, 1982). The study was carried out at saturating external free Ca^{2+} concentration (29 μM). A free internal Ca^{2+} concentration of 7.0 mM was observed for an ATP/(ADP*P_i) ratio of $10^{3.22}$. The free energy of the gradient was compared with that of ATP hydrolysis, and an efficiency of 49% was calculated. The lower than ideal efficiency was attributed to product inhibition of the pump by internal Ca^{2+} and to Ca^{2+} leakage mechanisms (Dixon *et al.*, 1982).

We have recently investigated the ability of the pump to establish and maintain a Ca^{2+} gradient as a function of Ca^{2+} concentration, pH, and other factors (Haynes and Mandveno, 1983). We observed that the pump can produce Ca^{2+} gradients of the order of 10^5, when studied at external Ca^{2+} concentrations equal to its K_m value. This can be compared with the gradients of the order of 10^3 observed in previous studies (Chiu and Haynes, 1980; Haynes and Mandveno, 1983) at saturating external Ca^{2+} concentrations. We concluded that the energetics of Ca^{2+} binding to the outwardly oriented translocator were a determinant factor in the gradient. We have now investigated the effect of ATP/(ADP*P_i) potential on the gradient under these conditions and report results identical to the theoretical predictions for 100% thermodynamic efficiency and tight coupling.

Materials and Methods

Active uptake of Ca^{2+} was examined in the low-density ATPase-rich fraction of rabbit skeletal sarcoplasmic reticulum. The SR vesicles were isolated according to the method of Chiu *et al.* (1980). Fluorescence of 1-anilino-8-naphthalenesulfonate (ANS) was used to indicate the free internal Ca^{2+} concentration ($[Ca^{2+}]_i$) in the SR lumen (Chiu and Haynes, 1980; Haynes and Mandveno, 1983).

The reaction medium contained 0.6 M sucrose, 20 mM Tris, 10 mM Hepes, pH 7.0, 1×10^{-4} M Mg^{2+}, 0.1 M KCl, and 1.5×10^{-6} M ANS. The free Ca^{2+} concentration was adjusted by the inclusion of 87 μM Ca^{2+} and 570 μM EGTA. This gives a free Ca^{2+} concentration of 4.7×10^{-8} M, using the

apparent binding constant of 3.77×10^6 M^{-1}, calculated as described previously (Haynes and Mandveno, 1983) using the intrinsic binding constants tabulated by Martell and Smith (1974). This free Ca^{2+} concentration is one-half the K_m of the pump under these conditions (Haynes and Mandveno, 1983). The rate of uptake and the maximal steady-state level at constant Ca^{2+}/EGTA ratio did not vary as a function of the total EGTA concentration.

Active uptake experiments were done with stopped-flow rapid mixing as described previously (Chiu and Haynes, 1980). The reaction was studied at 30°C, rather than 23°C as was done in the previous study (Dixon *et al.*, 1982). Both reservoirs contained the basic medium. One reservoir contained SR and valinomycin to give final concentrations of 0.1 mg/ml and 1.3×10^{-6} M, respectively, after mixing. The other reservoir contained Mg-ATP and P_i to give concentrations of 0.1 mM after mixing. This reservoir also contained a variable concentration of ADP. Extra Mg^{2+} was added with the ADP at a mole ratio of 0.057 such that the free Mg^{2+} concentration was not perturbed. In some experiments, the effect of the ATP/ADP ratio was studied in the absence of P_i. Active uptake was measured from the increase in ANS fluorescence observed after rapid mixing of the contents of the two reservoirs. The fluorescence change was displayed on an oscilloscope, and the amplitude of the total change was determined. The fluorescence change was converted into free internal Ca^{2+} concentration by use of a calibration curve obtained from passive Ca^{2+} jump experiments performed in the absence of ATP on the same day. The calibration data were used to transform the fluorescence change into $[Ca^{2+}]_i$ as described previously (Chiu and Haynes, 1980; Haynes and Mandveno, 1983).

Results and Discussion

We have studied the dependence of the free internal Ca^{2+} concentration established by the pump working at an external Ca^{2+} concentration (4.7×10^{-8} M) below its K_m value (7×10^{-8} M; Haynes and Mandveno, 1983) and at variable ATP/(ADP*P_i) ratio. Figure 1 shows the results of a typical experiment. The ATP and P_i concentrations were kept constant at 0.1 mM, and the ADP concentration was varied. The figure shows the dependence of $[Ca^{2+}]_i$ on the ATP/ADP ratio. At infinite ratio (absence of ADP) a level of 17 mM is observed. This is in fair agreement with previous observations (Haynes and Mandveno, 1983). Inclusion of ADP to give lower ATP/ADP ratios results in a decrease in $[Ca^{2+}]_i$. Similar results were observed in the absence of P_i, as described previously (Dixon *et al.*, 1982). Half-maximal uptake is observed for an ATP/(ADP*P_i) ratio of 3.5×10^3 M^{-1} (ATP/ADP

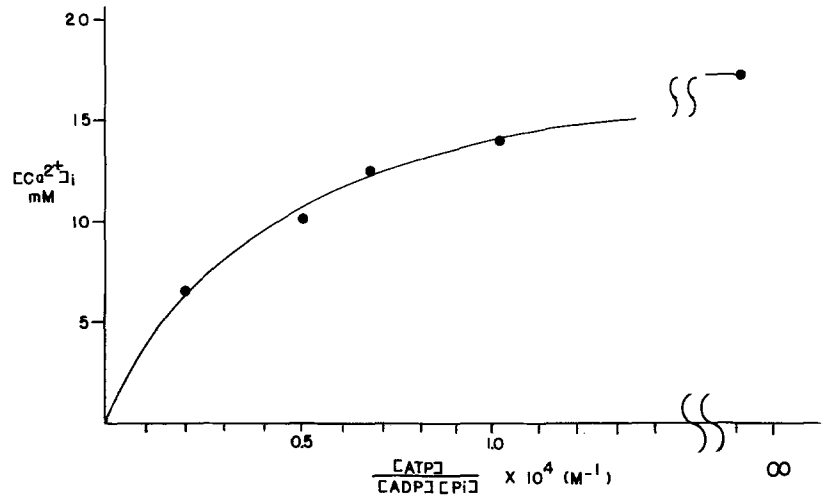

Fig. 1. Dependence of the free internal Ca^{2+} concentration on the ATP/ADP ratio. Active uptake experiments were carried out at 30°C as described under Materials and Methods. The Mg-ATP and P_i concentrations were fixed at 1×10^{-4} M and the ADP concentration was varied. Similar results were observed in the absence of P_i.

ratio = 0.35). This can be compared with our previous observation of half-maximal $[Ca^{2+}]_i$ at an ATP/ADP ratio of 0.17 (saturating external Ca^{2+} and $T = 23$°C). The finding of high values of $[Ca^{2+}]_i$ at comparable ATP/ADP ratios, but lower $[Ca^{2+}]_0$, indicates that the pump is capable of working at much higher efficiency than previously calculated.

We have previously shown that for an ideally coupled pump the overall reaction will be

$$2Ca_0^{2+} + ATP\ (+4\ Cl_0^-) \rightarrow 2Ca_i^{2+} + ADP + P_i\ (+4\ Cl_i^-) \qquad (1)$$

The Cl^- can be neglected as a determinant of the Ca^{2+} concentration since the ratio of its inside and outside concentration is close to 1 under all conditions. Thus the equilibrium constant for the reaction is given as:

$$K_{eq} = \frac{[Ca^{2+}]_i^2/[Ca^{2+}]_0^2}{[ATP]/([ADP][P_i])} \qquad (2)$$

The value of K_{eq} should be equal to the equilibrium constant for ATP hydrolysis. We have calculated this quantity for the present conditions. The free-energy of hydrolysis of ATP at 25°C, pH 7.0, in the presence of 0.1 mM Mg^{2+} is −9.4 kcal (Alberty, 1968; Shikama and Nakamura, 1973).[5] Using

[5]From more recent studies (Rosing and Slater, 1972; Guynn and Veech, 1973) a K_{eq} of $10^{5.8}$ can be estimated for our conditions. Rosing and Slater (1972) reported that the differences result from differences in the equilibrium constant of the glutamine synthetase reaction and in the stability constant of the magnesium glutamate complex. However, the assessment of both of these factors is complicated (cf. Guynn and Veech, 1973) and the earlier K_{eq} values will be used here.

$K_{eq} = 10^{(-\Delta G/2.303RT)}$ we get $K_{eq,25°C} = 7.84 \times 10^6$ M. The value for 30°C can be calculated using the enthalpy values of Phillips *et al.* (1969). For 0.1 mM Mg^{2+} and 0.1 M ionic strength, they gave values of −5.2, −4.9, and −4.7 kcal/mole for pH 6, 7.5, and 9, respectively. By linear interpolation between pH 6.0 and 7.5 we arrive at a value of −5.0 kcal/mole for our conditions. Using

$$\log(K_{eq,30°C}/K_{eq,25°C}) = \frac{-5.0}{(2.303)(1.987)}(1/303 - 1/298) \qquad (3)$$

we arrive at $K_{eq,30°C}/K_{eq,25°C} = 1.15$. This gives a value of 9.02×10^6 for $K_{eq,30°C}$.

Equation (2) predicts that a plot of the square of the Ca^{2+} gradient against the ATP/(ADP*P_i) ratio should give a straight line with a slope equal to K_{eq}. Figure 2 is a plot of the data of Fig. 1 according to this assumption. The solid line is the theoretical result (slope = 9.02×10^6 M) under the assumption of perfect coupling and 100% pump efficiency. The agreement between the theory and experiment is excellent. The comparison depends upon three factors, the equilibrium constant for ATP hydrolysis, the Ca^{2+}/EGTA dissociation constant, and measurement of the free internal Ca^{2+} concentra-

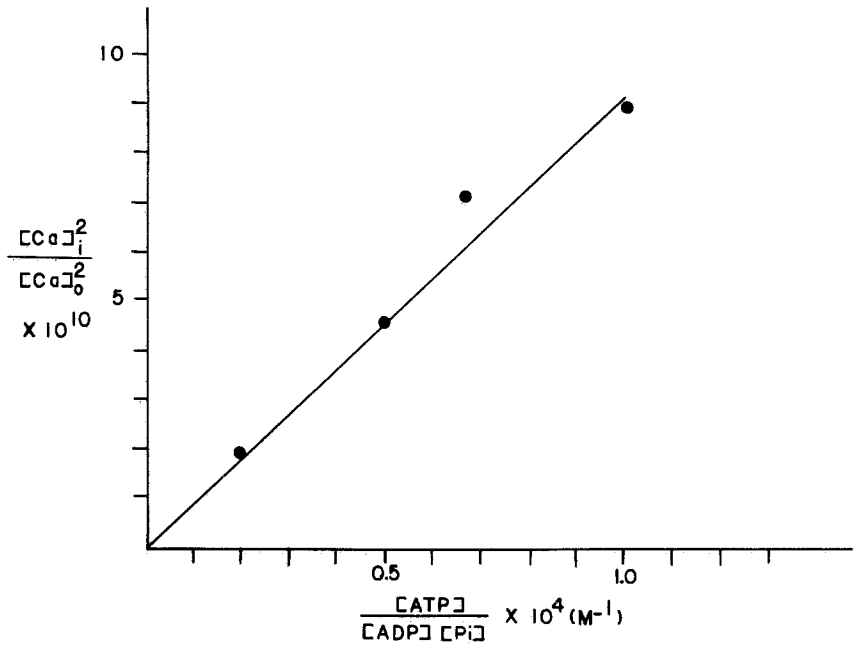

Fig. 2. Comparison of experimental and theoretical dependence of Ca^{2+} gradient on ATP/(ADP*P_i) ratio. The experimental data are the first four points of Fig. 1. The fifth point at infinite ATP/(ADP*P_i) ratio was omitted. The straight line is a theoretical line with slope = 9.02×10^6 M.

tion. The latter quantity was the most subject to experimental error, with approximately 20% variation from preparation to preparation or day to day. Taking this into consideration, we calculate the efficiency of the pump as $100 \pm 3\%$.

Our results show that the intrinsic efficiency of the pump is 100% and this can be measured for $[Ca^{2+}]_0 < K_m$. Our previous study (Dixon *et al.*, 1982) showed a lower efficiency is measured for $[Ca^{2+}]_0 \gg K_m$. The differences can be reconciled if it is recognized that the internal Ca^{2+} level achieved is dependent on the characteristics of both the pump and the passive leak. Ideal thermodynamic efficiency will be measured when the ability of the pump to move Ca^{2+} is high with respect to the ability of the passive leak to remove it. The gradient obtained in this case will be determined by the energetics of the pump, i.e., the ATP/(ADP*P_i) ratio. Conversely, less than ideal efficiency will be observed when the ability of the pump to move Ca^{2+} is only comparable to the ability of the passive leak to remove it. We have shown that the rate of Ca^{2+} uptake decreases with increasing $[Ca^{2+}]_i$ (pump inhibition) and that the rate of leakage ($k_{leak}[Ca^{2+}]_i$) is proportional to $[Ca^{2+}]_i$ (Haynes and Mandveno, 1983). The maximal $[Ca^{2+}]_i$ achievable will be obtained at $[Ca^{2+}]_i$ levels which are sufficient to inhibit the pump such that its rate is equal to the passive leak rate. This occurs at moderate ATP/(ADP*P_i) ratios for $[Ca^{2+}]_0 \gg K_m$ (Dixon *et al.*, 1982) and at infinite ATP/(ADP*P_i) ratio for $[Ca^{2+}]_0 < K_m$ ([ADP] = 0 in the present study). Under these conditions, less than optimal efficiency will be measured. Conversely, for moderate ATP/(ADP*P_i) ratios and $[Ca^{2+}]_0 < K_m$, the $[Ca^{2+}]_i$ is not sufficiently high for the pump to be inhibited and for the leak to express its influence, and theoretical efficiency values are obtained. Optimal thermodynamic efficiency will be observed when all three of the following processes are determinative factors in the formation of the gradient: (1) association with the outwardly oriented translocator ($[Ca^{2+}]_0 < K_m$), (2) the inside vs. outside orientation of the loaded translocators [ATP/(ADP*P_i) ratio], and (3) dissociation from the inwardly oriented translocator ($[Ca^{2+}]_i < K_i$, the inhibitory constant). We believe that the knowledge of the overall equilibrium constant of the pump will prove useful in establishing the equilibrium constants of several steps of the enzyme cycle which are as yet experimentally inaccessible (translocation and countertranslocation).

The present results are pertinent to the energetics of the skeletal muscle.[6] They predict that the Ca^{2+} gradient across the SR membrane will be in thermodynamic equilibrium in the resting condition (cytoplasmic Ca^{2+} con-

[6]Tanford used data (Hasselbach, 1979; Martonosi, 1980) on the Ca^{2+} level in the SR of resting calls, various estimates, estimates of the intracellular Ca^{2+} concentration, and an estimate of the ATP/ADP/P_i potential (Veech *et al.*, 1979) to conclude that the uptake reaction is "limited by the thermodynamics of the overall reaction" under physiological conditions.

centration less than K_m) and that energy will be wasted if the pump is continuously presented with saturating cytoplasmic Ca^{2+} concentrations as it would in a continuously activated muscle. The latter might be important in acute conditions such as muscle fatigue, cramp, ischemia, or malignant hyperthermia.

Acknowledgments

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