Entropic Drive in the Sarcoplasmic Reticulum ATPase Interaction with Mg^{2+} and Pi

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ABSTRACT Thermodynamic quantities for the binding of Mg^{2+} (in the presence of Ca^{2+}) and Pi (in the presence of Mg^{2+} and absence of Ca^{2+}) to sarcoplasmic reticulum ATPase were determined from isothermal titration calorimetry measurements at 25°C. Mg^{2+} and Pi are involved in reversal of the ATPase hydrolytic reaction, and their interactions with the ATPase are conveniently studied under equilibrium conditions. We found that the Mg^{2+} binding reaction is endothermic with a binding constant (K_b) = 142 ± 4 M^{-1}, a binding enthalpy of 180 ± 3 kJ mol^{-1}, and an entropy contribution ($T\Delta S_b$) = 192 ± 3 kJ mol^{-1}. Similarly, Pi binding is also an endothermic reaction with K_b = 167 ± 17 M^{-1}, ΔH_b = 65.3 ± 5.4 kJ mol^{-1}, and $T\Delta S_b$ = 77.9 ± 5.4 kJ mol^{-1}. Our measurements demonstrate that the ATPase can absorb heat from the environment upon ligand binding, and emphasize the important role of entropic mechanisms in energy transduction by this enzyme.

INTRODUCTION

[1]An interesting property of the sarcoplasmic reticulum (SR) ATPase is its ability to react with Pi in the presence of Mg^{2+} and in the absence of Ca^{2+} (Masuda and deMeis, 1973). The Pi reaction involves an aspartyl residue, and the resulting phosphoenzyme corresponds to a catalytic intermediate that is otherwise formed by utilization of ATP in the presence of Ca^{2+} (Fig. 1). Considering that a reaction of Pi with aspartate in aqueous solution would be highly unfavorable (Jencks, 1980), it is likely that phosphorylation of the ATPase aspartyl residue is linked to enzyme isomerization. To clarify the energetic implications of putative isomerizations induced by ligands, we report here thermodynamic parameters obtained by isothermal titration calorimetry (ITC). Mg^{2+} (Fig. 1 *A*) and Pi (reversal of reaction 4 in Fig. 1 *B*) were chosen as they are specific ligands in the ATPase cycle and can be used for ATPase titrations under convenient equilibrium experimental conditions.

EXPERIMENTAL PROCEDURES

Materials

SR vesicles were obtained from rabbit skeletal muscle as previously described (Eletr and Inesi, 1972a,b). All other reagents were analytical grade from Sigma Chemical Co. (St. Louis, MO).

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Certain commercial equipment, instruments, and materials are identified in this paper to specify the experimental procedure as completely as possible. In no case does this identification imply a recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the material, instrument, or equipment identified is necessarily the best available for the purpose.

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Mg^{2+} titrations

Glassware was soaked overnight in 1 mM ethylene diaminetetraacetic acid (EDTA) in deionized water and then rinsed several times with deionized water. Two liters of the buffer was prepared in deionized water and stored over 15 g of chelex for 48 h. The buffer consisted of 30 (m/m) % sucrose, 30 mM MOPS, and 80 mM KCl buffered at pH 7.2 (MOPS buffer). Before the dialysis, dialyzing tubing and clips were treated by placing them in 1 mM EDTA solution and heating the solution up to 95°C for approximately 30 min. The protein suspension was dialyzed in 1 L of the buffer over 0.5 g of chelex overnight. $CaCl_2$ was added by weight separately to the protein suspension and to the dialysate to make up 0.1 mM Ca^{2+} solutions. For the Mg^{2+} ligand solution, $MgCl_2$ was then added to the Ca^{2+}-dialysate. Before the titrations, the titration cell, the titration syringe, and the syringe used for filling the cell were extensively rinsed with deionized water.

Phosphate titrations

The protein suspensions were dialyzed overnight in 25 mM 2-[*N*-morpholino]ethane-sulfonic acid (MES)-tris(hydroxymethyl)aminoethane buffer (TRIS) at pH 6.2 containing 10 mM $MgCl_2$, 2 mM ethylene glycol-bis(β-aminoethylether)-*N*, *N*, *N*, *N*′tetraacetic acid (EGTA), and 30 (m/m) % sucrose (MES-TRIS buffer). The phosphate ligand solution was 100 mM H_3PO_4 in TRIS buffer at pH 6.2 and contained 30 (m/m) % sucrose (TRIS buffer). No heat was observed upon titrating the phosphate ligand solution into the dialysate from the protein dialysis, indicating that the omission of EGTA and $MgCl_2$ from the ligand solution would not affect the ITC measurements.

ITC measurements and analysis

ITC measurements were performed with a Microcal Omega titration calorimeter as described previously (Wiseman et al., 1989; Schwarz et al., 1991). Aliquots of the ligand solution at 10–20X the site concentration were added via a 100-μl rotating stirrer-syringe to the solution cell containing 1.34 ml of the 26.4–37.6 mg/ml ATPase suspension. The heat of dilution for the Mg^{2+} and PO_3^{-3} ligand solutions determined by titration of the ligand into the buffer solution was subtracted from the heat observed upon addition of the ligand solution to the protein suspension.

The total heat content, Q_t, is related to the total ligand concentration,

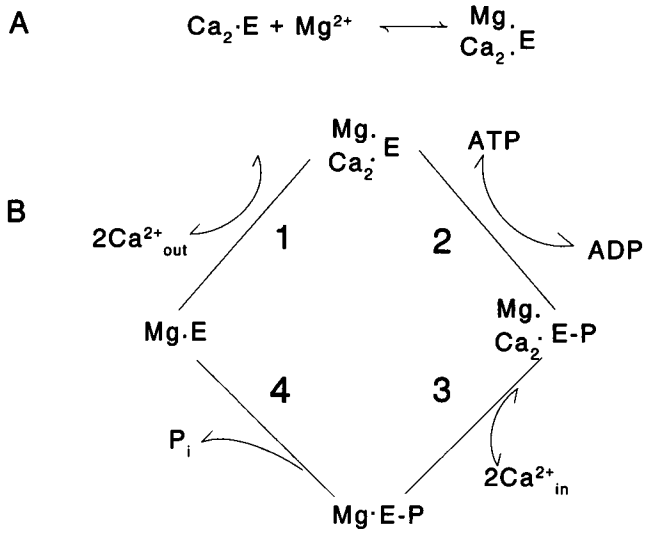

FIGURE 1 Reaction schemes for Mg^{2+} binding to SR ATPase in the presence of Ca^{2+} (*A*) and for the ATPase catalytic and transport cycle (*B*). Enzyme phosphorylation with Pi is the reversal of partial reaction 4 of the ATPase cycle. E, enzyme (i.e., ATPase). *A* and *4B* are the reactions studied by our calorimetric measurements.

$[L]_t$, via the following equation (Wiseman et al., 1989):

$$Q_t = n[\text{ATPase}]_t \Delta H_b V\{1 + [L]_t/n[\text{ATPase}]_t + 1/nK_b[\text{ATPase}]_t - [(1 + [L]_t/n[\text{ATPase}]_t + 1/nK_b[\text{ATPase}]_t)^2 - 4[L]_t/n[\text{ATPase}]_t]^{1/2}\}/2 \quad (1)$$

where n is the stoichiometry, [ATPase] is the protein concentration, and V is the cell volume. The expression for the heat released per the ith injection, $\Delta Q(I)$, is then (Yang, 1990)

$$\Delta Q(I) = Q(I) + dV_i/2V[Q(I) + Q(I-1)] - Q(I-1) \quad (3)$$

where dV_i is the volume of titrant added to the solution.

The thermodynamic quantities, ΔG_b^0 and ΔS_b, were obtained from the basic equation of thermodynamics,

$$\Delta G_b^0 = \Delta H_b - T\Delta S_b \quad (4a)$$

where

$$\Delta G_b^0 = -nRT \ln\{K_b\} \quad (4b)$$

and n = number of moles, T is in degrees Kelvin, and R = 8.3151 J mol^{-1} K^{-1}.

RESULTS

Mg^{2+} is an absolute requirement for SR ATPase phosphorylation in the absence of Ca^{2+} as well as for ATP utilization by the SR ATPase in the presence of Ca^{2+}. In fact, Mg^{2+} activates the SR ATPase by forming a stoichiometric complex and remains bound to the enzyme throughout the catalytic cycle (Coll and Murphy, 1992; Coan et al, 1993). We have titrated the ATPase with Mg^{2+} in the presence of Ca^{2+} to minimize divalent cation effects that would be nonspecific for Mg^{2+} (i.e., nonspecific binding to Ca^{2+} sites). The results of the ITC are shown in Fig. 2. It is clear that the Mg^{2+} binding reaction is endothermic. The derived thermodynamic quantities are as follows: K_b, = 142 ± 4 M^{-1}, ΔH_b = 180 ± 3 kJ mol^{-1}, ΔG^0 = −12.2 ± 0.1 kJ mol^{-1}, and $T\Delta S_b$ = 192 ± 3 kJ mol^{-1}.

Phosphorylation of the SR ATPase by Pi requires the presence of Mg^{2+} and the absence of Ca^{2+} and is favored by a slightly acid pH (Masuda and de Meis, 1973; de Meis and Masuda, 1974). The isothermal calorimetric curve derived by ATPase titration with Pi under these conditions is shown in Fig. 3. The Pi reaction is endothermic, with K_b = 167 ± 17 M^{-1} in agreement with the Pi concentration dependencies observed by chemical measurements (Puntzengruber et al., 1978). ΔH_b is 65.3 ± 5.4 kJ mol^{-1}, and therefore the reaction is entropically driven. Considering that $\Delta G_b°$ is −12.6 ± 0.3 kJ mol^{-1} (derived from K_b), $T\Delta S_b$ is then 77.9 + ± 5.4 kJ mol^{-1}.

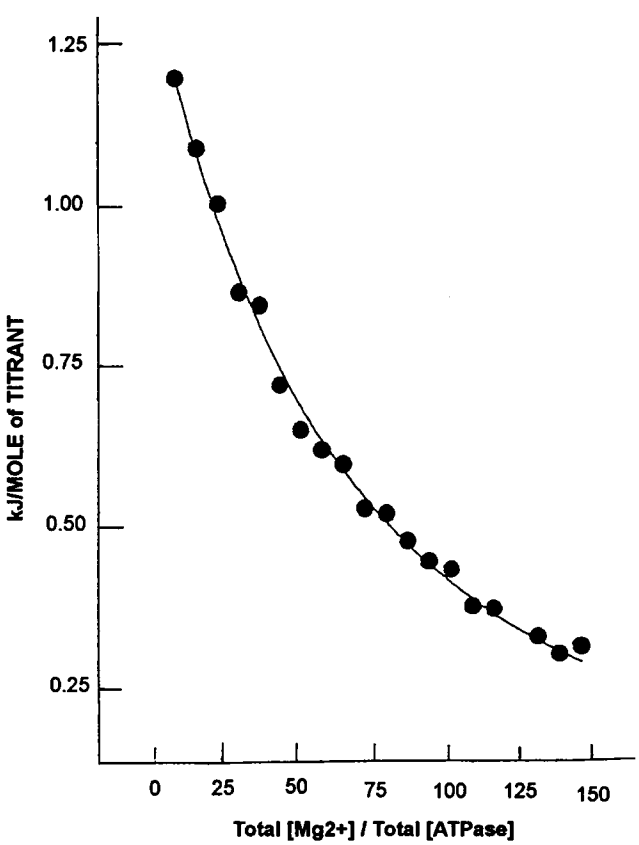

FIGURE 2 The binding isotherm from a titration of 5-μl aliquots of 100 mM $MgCl_2$ into 0.042 mM ATPase at 23.2°C. The protein suspension (sarcoplasmic reticulum vesicles) was dialyzed overnight in 30 mM MOPS, pH 7.2, 80 mM KCl, and 30 (m/m) % sucrose over Chelex. $CaCl_2$ was then added separately to the protein suspension and to the dialysate to make up a 0.1 mM concentration. $MgCl_2$ was then added to the dialysate to make up the 100 mM ligand solution.

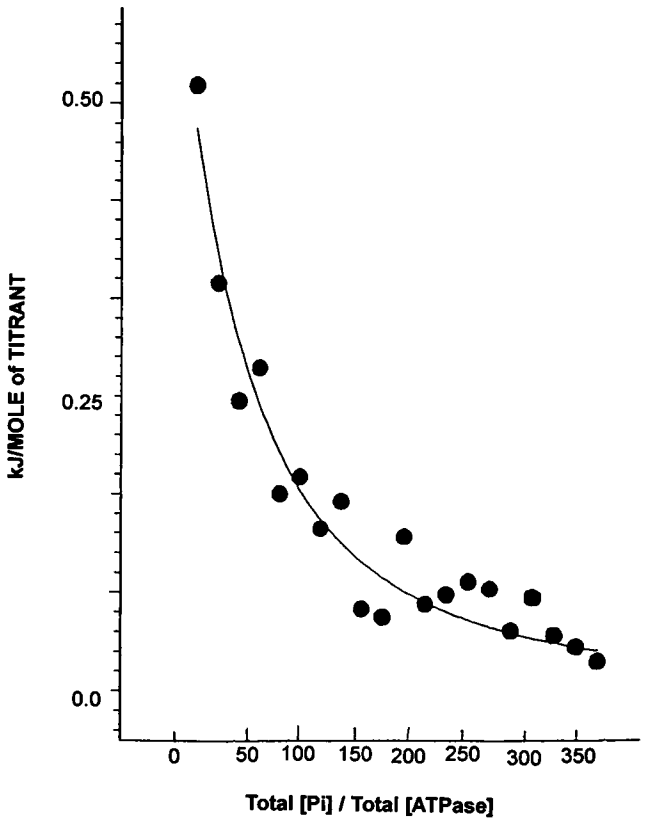

FIGURE 3 The binding isotherm from a titration of 10-μl aliquots of 100 mM Pi into 0.049 mM ATPase at 24.2°C. The protein suspension (sarcoplasmic reticulum vesicles) was dialyzed overnight in 25 mM MES-TRIS buffer, pH 6.2, 10 mM $MgCl_2$, 2 mM EGTA, and 30 (m/m) % sucrose. The ligand solution contained 100 mM Pi, 25 mM MES-TRIS buffer, pH 6.2, and 30 (m/m) % sucrose. No heat was observed upon titrating the Pi ligand solution into the dialysate containing no protein.

DISCUSSION

Our calorimetric titrations demonstrate that Mg^{2+} and Pi associations with the SR ATPase are endothermic. The resulting endothermic heat is quite large and, just for Mg^{2+}, corresponds to approximately one-third of the unfolding heat for a small globular protein such as lysozyme. It should be understood that flow and balance of potential energy from ATP for the overall catalytic and transport cycle have been already accounted for in detail, based on the equilibrium constants of partial reactions (Inesi, 1985, 1994; Pickart and Jencks, 1984). Calorimetric measurements in this regard have been also reported (Kodama et al., 1982; deMeis et al., 1997). On the other hand, our present measurements demonstrate that the ATPase protein can absorb heat from the environment. Specifically, the endothermic heat triggered by Mg^{2+} and Pi binding provides an entropic drive for the phosphorylation reaction with Pi, as also suggested by studies of the temperature dependence of the Pi reaction (Kanazawa, 1975). More generally, our demonstration that the SR ATPase is able to absorb heat from the environment upon binding specific ligands suggests that entropic drive plays a role in the mechanism of the SR ATPase and other energy-transducing enzymes.

As for a structural counterpart to the entropy changes, it should be pointed out that only very small changes in secondary structure follow ligand binding and catalytic activation of the SR ATPase (Nakamoto and Inesi, 1986; Barth et al., 1996). Therefore, the large endothermic heat detected by our calorimetric titrations must be related to segmental motion of secondary structural units and tertiary structural elements (Tanford, 1982) and/or dissociation of water molecules (deMeis, 1989). For instance, Mg^{2+}-dependent changes in the electron density profile of the SR ATPase have been observed by x-ray diffraction (Asturias and Blasie, 1989). In addition, ATPase oligomerization has been shown by spectroscopic methods (Mahaney et al., 1995), and dissociation of oligomers was observed upon ATPase phosphorylation (Watanabe and Inesi, 1982; Bigelow et al., 1992). It is also of interest that the Pi reaction is strongly influenced by the presence of solvents that are likely to favor water dissociation from the ATPase (deMeis et al., 1980).

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