Calorimetric Studies of Ligand-Induced Modulation of Calcium Adenosine 5′-Triphosphatase from Sarcoplasmic Reticulum†

Michael Epstein,‡ Yoshitaka Kuriki,§ Rodney Biltonen, and Efraim Racker*

ABSTRACT: The enthalpy change ($\Delta H°'$) associated with the binding of Mg^{2+} to the sarcoplasmic reticulum calcium adenosine 5′-triphosphatase [(Ca^{2+})ATPase] is −76 kcal/mol. The affinity constant for Mg^{2+} obtained from calorimetric measurements agrees with the K_m value for Mg^{2+} in the phosphorylation of the enzyme by inorganic phosphate (P_i). The $\Delta H°'$ of binding of P_i to the enzyme is −23.5 kcal/mol, and the affinity constant for P_i obtained from the calorimetry also agrees with the K_m value for P_i in the phosphorylation reaction. $\Delta H°'$ of Mg^{2+} binding is reduced to −35 kcal/mol in the presence of either 20 mM P_i or 1.2 mM Ca^{2+} without a significant change in the affinity of the enzyme for Mg^{2+}. $\Delta H°'$ of P_i binding to the enzyme drops to −8.5 kcal/mol in the presence of 10 mM Mg^{2+} without a significant change in the affinity of the enzyme for P_i. On the other hand, the presence of Ca^{2+} does not affect the $\Delta H°'$ for the binding of the substrate analogue 5′-adenylyl β,γ-imidodiphosphate [App(NH)p], and the presence of this analogue does not affect the $\Delta H°'$ for Ca^{2+} binding. The results suggest a model in which a conformational change, largely controlled by Mg^{2+} binding to the enzyme, leads to the formation of the covalent phosphoprotein intermediate.

Plasma membrane (Na^+/K^+)ATPase and (Ca^+)ATPase of the sarcoplasmic reticulum form phosphorylated intermediates under appropriate conditions with either ATP or P_i[1] as substrates (Albers et al., 1963, 1968; Yamamoto & Tonomura, 1967; Lindenmeyer et al., 1968; Masuda & de Meis, 1973). The phosphate is covalently bound to a carboxyl group of an aspartic acid residue of the protein (Degani & Boyer, 1973; Post & Orcutt, 1973). The phosphorylated enzyme intermediate is capable of transferring stoichiometrically its covalently bound P_i to ADP to form ATP (Taniguchi & Post, 1975; Knowles & Racker, 1975).

Ca^{2+} is necessary for the phosphorylation of the enzyme by ATP (Panet et al., 1971; Garrahan et al., 1976) and for the phosphorylation of ADP by the phosphoenzyme (Knowles & Racker, 1975) and is inhibitory to the phosphorylation of the enzyme by P_i (Kanazawa, 1975; Knowles & Racker, 1975). Mg^{2+} is necessary for the enzyme phosphorylation by P_i and for dephosphorylation of phosphoenzyme through release of P_i into solution (Panet et al., 1971; Garrahan et al., 1976).

In a recent paper (Kuriki et al., 1976), we reported the results of a calorimetric study of the binding of P_i and Mg^{2+} to the (Na^+/K^+)ATPase. These studies revealed that both ions bound to the enzyme with an extraordinarily large enthalpy change; $\Delta H°' = -49$ kcal/mol for Mg^{2+} and $\Delta H°' = -42$ kcal/mol for P_i. In the presence of P_i, the binding of Mg^{2+} was accompanied by a reduced enthalpy change. These and other findings (e.g., changes in reactivity with borohydrate) indicated that Mg^{2+} induced a thermodynamically significant conformation change in the enzyme and that the new form of the protein is one which can be phosphorylated.

These studies have now been extended to the (Ca^{2+})ATPase isolated from sarcoplasmic reticulum. The interaction of the enzyme with Mg^{2+} or P_i again produced large enthalpy changes, but distinct differences in the magnitude of the change were noted. Furthermore, Ca^{2+} binding also produced a large enthalpy change. The reactions of Ca^{2+}, Mg^{2+}, and P_i with the enzyme appear to be thermodynamically coupled, but the binding of Ca^{2+} and of the substrate analogue App(NH)p appears to be independent of each other. The results are consistent with a model of ion transport controlled by ion binding to the enzyme system.

Experimental Procedures

$MgCl_2$ solutions were prepared from a 1 M commercial solution in 10^{-2} M HCl (Sigma Chemical Co., St. Louis, MO) by mixing with a concentrated, basic imidazole solution. The pH of this solution was adjusted to 6.2 and diluted to the desired concentration of Mg^{2+} and a final concentration of imidazole equal to 40 mM.

(Ca^{2+})ATPase was prepared from sarcoplasmic reticulum according to MacLennan (1970), with the exception that the extraction buffer was 5 mM Hepes (pH 7.5), 0.12 M NaCl, and 0.5 mM phenylmethanesulfonyl fluoride. The storage buffer was 5 mM Hepes (pH 7.5) and 0.3 M sucrose. The preparation was stored at −70 °C at a concentration of ~25 mg/mL. It had an activity of about ~15 units/mg (retained for over 6 months) and a phospholipid content of 0.5 μmol of P/mg of protein.

The Ca^{2+}-dependent ATPase activity was assayed for 4 min at 37° C with [γ-^{32}P]ATP as described by MacLennan (1970). After addition of 10% (final concentration) trichloroacetic acid and centrifugation, the $^{32}P_i$ formed was extracted from the supernatant according to Martin & Doty (1949) and counted by liquid scintillation.

Phosphorylation of (Ca^{2+})ATPase by P_i was measured in an 0.5-mL reaction mixture containing 40 mM imidazole–HCl (pH 6.0) and 0.5 mg of (Ca^{2+})ATPase containing varying amounts of $MgCl_2$ and Tris–$^{32}P_i$ (10^7 cpm/μmol, pH 6). In the absence of detergent and at low salt concentration, pH 6 was shown to be optimal for the phosphorylation of (Ca^{2+})-

† From the Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853 (M.E., Y.K., and E.R.), and the Departments of Biochemistry and Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22908 (R.B.).
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‡ Present address: The Rogoff-Wellcome Research Institute, The Beilinson Medical Center, Petah Tikva, Israel.
§ Present address: Institute for Protein Research, Osaka University, Suita, Osaka, Japan.

[1] Abbreviations used: P_i, inorganic phosphate; ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; App(NH)p or AMPPNP, 5′-adenylyl β,γ-imidodiphosphate.

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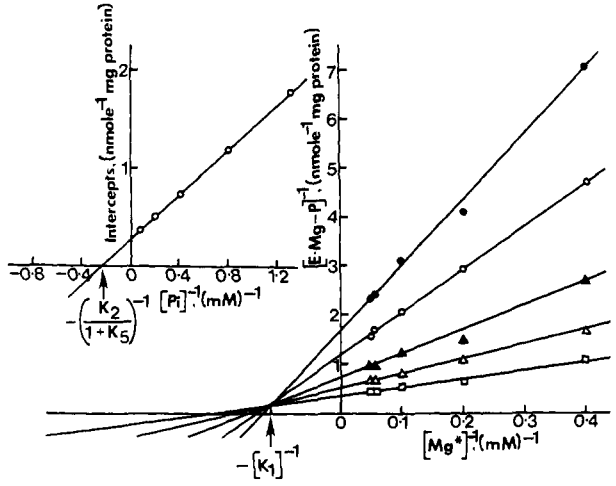


FIGURE 1: Dependence of the amount of phosphoenzyme formed on Mg^{2+} concentration at various P_i concentrations. (●) 0.76; (○) 1.25; (▲) 2.5; (△) 5; (□) 12.5 mM P_i. The reaction mixture, 0.5 mL containing 40 mM imidazole, pH 6.0, $^{32}P_i$–Tris at pH 6 ($\sim 10^7$ cpm/mol) and $MgCl_2$ both at the concentrations indicated, and 0.5 mg of (Ca^{2+})ATPase, was incubated at room temperature (22–24 °C) for 10 min. An aliquot of 0.5 mL of 5 N trichloroacetic acid containing 20 mM P_i was added to stop the reaction. The filtration and washing of the precipitate and the determination of the amount of phosphoenzyme formed were as described by Kuriki et al. (1976).

ATPase from P_i (Knowles & Racker, 1975). After a 10-min incubation at room temperature, a time sufficient to attain equilibrium (Kanazawa, 1975; Boyer et al., 1977), the reaction was stopped by addition of 0.5 mL of cold 5 N trichloroacetic acid. The precipitate was treated as described by Kuriki et al. (1976).

Calorimetric measurements were performed with an LKB batch microcalorimeter at 24.5 °C. For each day of experiments, an aliquot of (Ca^{2+})ATPase containing 32 mg of enzyme was diluted to 50 mL with the standard imidazole buffer (40 mM, pH 6.2) and centrifuged for 20 min at 40000 rpm in a Spinco 60 Ti rotor (average of 113000*g*). The pellet was suspended with a tight, all-glass homogenizer in the same buffer to yield a concentration of ~3 mg/mL. The procedure of the calorimetric measurement was as described by Kuriki et al. (1976) except that the thermal equilibration time was 1 h.

Results

Phosphorylation of (Ca^{2+})ATPase with P_i. Phosphoenzyme formation was measured as a function of [P_i] at constant [Mg^{2+}] and as a function of [Mg^{2+}] at constant [P_i]. In both cases, a linear reciprocal relationship between the amount of phosphoenzyme formed and the concentration of the variable ion was found as shown in Figures 1 and 2. The extrapolated values (infinite Mg^{2+} and P_i) of phosphoenzyme formed, [E–P]$_{max}$, were 3.6 nmol/mg of protein (Figure 1) and 4.0 nmol/mg of protein (Figure 2). These values represent phosphorylation of 30–35% of the protein assuming 98% purity (MacLennan, 1970) and a molecular weight of 115000 (Dean & Tanford, 1977). Although these values are similar to those observed with detergent-solubilized sarcoplasmic reticulum vesicles at 37 °C and pH 7 (Kanazawa, 1975), this may be coincidental. The presence of detergent and pH 7 (compared to pH 6 used in our study with the pure enzyme) reduce the amount of phosphorylated enzyme, while the higher temperature (37 °C compared to 24.5 °C used in our study) increases it (Kanazawa, 1975).

These results are consistent with Scheme I in which the phosphorylation of the enzyme is preceded by formation of

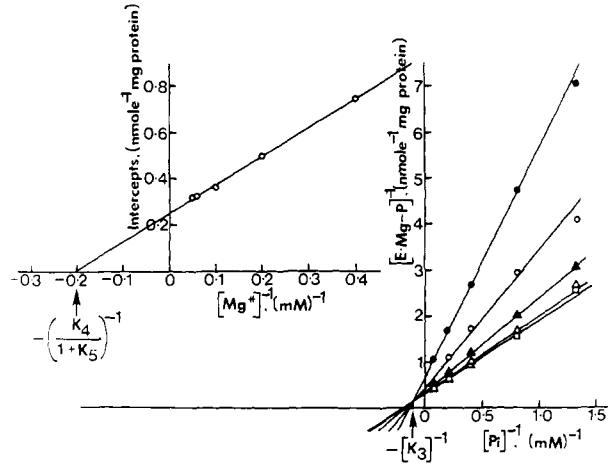
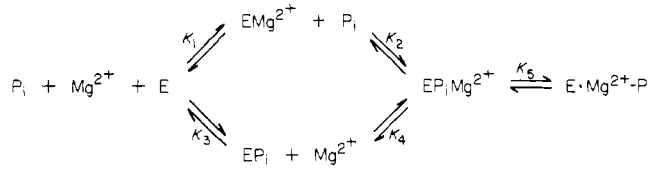


FIGURE 2: Dependence of the amount of phosphoenzyme formed on P_i concentration at various Mg^{2+} concentrations. (●) 2.5; (○) 5; (▲) 10; (△) 17.5; (□) 25 mM Mg^{2+}. The experimental conditions are the same as in Figure 1.

Scheme I



an obligatory ternary complex of ATPase, P_i, and Mg^{2+}. The mathematical relationship between the degree of phosphorylation, the various equilibrium constants, [P_i], and [Mg^{2+}] is given by eq 1 where K_1, K_2, K_3, and K_4 represent the disso-

$$[E\cdot Mg-P] = \frac{[Mg^{2+}][P_i][E_{total}]K_5}{K_1K_2 + K_2[Mg^{2+}] + K_4[P_i] + (1 + K_5)[Mg^{2+}][P_i]} \quad (1)$$

ciation constants for Mg^{2+} or P_i at each step shown in the scheme. K_5 represents the equilibrium constant for formation of the phosphoenzyme from the ternary complex of Mg^{2+}, P_i, and the enzyme.

The values of the equilibrium constants obtained from analysis of the data shown in Figures 1 and 2 are tabulated in Table I. K_5, the equilibrium constant between the phosphoenzyme and the ternary complex of enzyme, Mg^{2+}, and P_i, was calculated from the relationship

$$K_5 = \frac{[E-P]_{max}}{[E]_{total} - [E-P]_{max}} \quad (2)$$

assuming the molecular weight of the enzyme is 115000 (Rizzolo et al., 1976; Dean & Tanford, 1977).

Calorimetric Studies of Ion Binding. The calorimetric experiments were performed at 24.6 ± 0.1 °C by mixing 2 mL of a 3 mg/mL ATPase solution with 4 mL of ligand dissolved in the same buffer. The measured heat of mixing includes the heat of dilution of the various components plus the apparent heat of interaction of the ligand with the protein. The heat of dilution of the ligand was balanced out by an equivalent dilution experiment being carried out simultaneously in the reference cell of the calorimeter. The heat of dilution of the protein in the absence of ligand was measured in a separate experiment and subtracted from the measured heat. This value was 0–5 kcal/mol of protein. The resulting heat effect thus represents the apparent enthalpy change ($\Delta H^{\circ\prime}$) associated with ligand binding to the protein.

Table I: Equilibrium Constants and Apparent Thermodynamic Quantities for the Interaction of Various Ions with (Ca^{2+})ATPase[a]

reaction	phosphorylation results, K_d	calorimetric results			
		$K_d \times 10^{-3}$ M	$-\Delta H^{\circ\prime}$ (kcal/mol)	$-\Delta G^{\circ\prime}$ (kcal/mol)	$-\Delta S^{\circ\prime}$ (eu)
$E + Mg^{2+} \rightleftharpoons EMg^{2+}$	$K_1 = 9 \times 10^{-3}$ M	7	76	3.0	245
$EMg^{2+} + P_i \rightleftharpoons EMg^{2+}P_i$	$K_2 = 8 \times 10^{-3}$ M	6	9	3.0	20
$E + P_i \rightleftharpoons EP_i$	$K_3 = 10 \times 10^{-3}$ M	9	23	2.8	67
$EP_i + Mg^{2+} \rightleftharpoons EMg^{2+}P_i$	$K_4 = 9 \times 10^{-3}$ M	5	35	3.2	107
$EMg^{2+}P_i \rightleftharpoons EMg^{2+}$-P	$K_5 = 0.8$				
$E + Ca^{2+} \rightleftharpoons ECa^{2+}$		0.018	24	6.6	58
E + App(NH)p $\rightleftharpoons$ EApp(NH)p		0.006	34	7.3	90
$E + Mg^{2+} \rightleftharpoons EMg^{2+}$ (1.2 mM Ca^{2+})		6	35	3.1	107

[a] E denotes the enzyme. $\Delta H^{\circ\prime}$, $\Delta G^{\circ\prime}$, and $\Delta S^{\circ\prime}$ were related to the association reaction and were calculated by assuming a standard of 1 M.

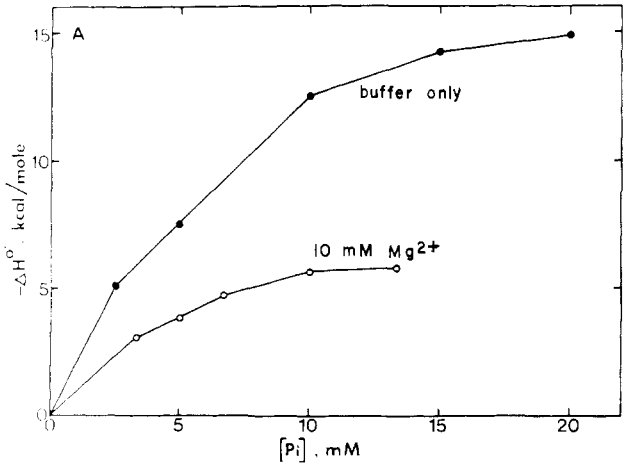


FIGURE 3: The enthalpy isotherm of inorganic phosphate binding to the (Ca^{2+})ATPase in the absence of (●) and in the presence of (○) 10 mM Mg^{2+}. The calorimetric measurements are described in the text.

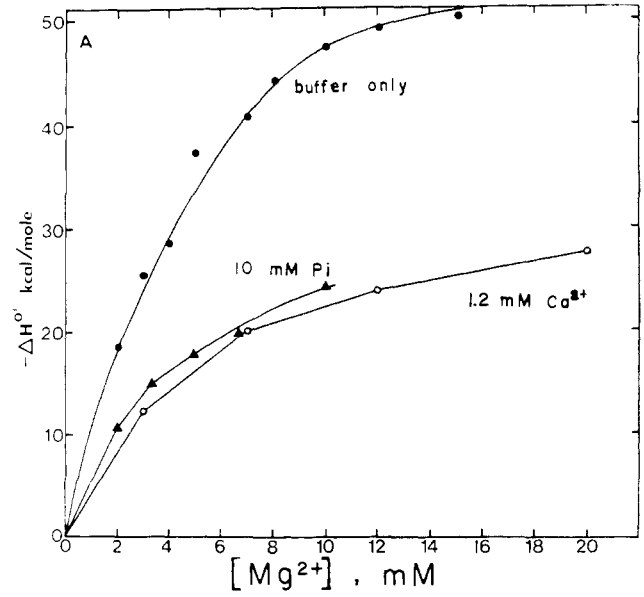


FIGURE 4: The enthalpy isotherm of Mg^{2+} binding to the (Ca^{2+})-ATPase in the absence (●) and in the presence of 10 mM P_i (▲) and 1.2 mM Ca^{2+} (○). The calorimetric measurements are described in the text.

These data were transformed into reciprocal heat units and ligand concentration and analyzed by a weighted, linear least-squares procedure to provide estimates of the maximum heat change and the apparent binding constant of the ligand to the protein system. The details of the analytical procedure have been described by Bolen et al. (1971). In all experiments, the resulting double-repciprocal plot was found to be linear, which is consistent with the existence of a single set of equivalent and mutually independent binding sites. The errors in the estimated parameters, $\Delta H^{\circ\prime}$ and K, are judged to be about ±10% and ±30%, respectively.

The apparent heat of interaction of P_i with the (Ca^{2+})ATPase was measured over a range of 1–20 mM final P_i concentration. The resulting enthalpy isotherm is shown in Figure 3. The best estimate of $\Delta H^{\circ\prime}$ was −23 kcal/mol and $K_d = 9 \times 10^{-3}$ M. The agreement with the value obtained from the phosphorylation measurements, $K_d = 10 \times 10^{-3}$ M, is consistent with the outlined scheme.

A similar calorimetric experiment was carried out in the absence of P_i with Mg^{2+} as the ligand. Analysis of the data, shown in Figure 4, yielded parameters of $\Delta H^{\circ\prime} = -76$ kcal/mol and $K_d = 7 \times 10^{-3}$ M. This same experiment was performed with several preparations of enzyme, including a few in which 10^{-4} M Ca^{2+} was added at the step of solubilization with deoxycholate (MacLennan, 1970). Analysis of the data yielded values of $\Delta H^{\circ\prime} = -78$ kcal/mol and $K_d = 10 \times 10^{-3}$ M. These values agree, within experimental error, with the value of $K_d = 9 \times 10^{-3}$ M obtained from the phosphorylation measurements. The absolute value of $\Delta H^{\circ\prime}$ varied from preparation to preparation. This variation appeared to be correlated with the efficiency of removal of residual ions, exact phospholipid content, and specific activity. In all cases, however, within a given preparation, the estimated value of $\Delta H^{\circ\prime}$ of binding of each of the ligands remained constant. The $\Delta H^{\circ\prime}$ values reported in Table I are those obtained from a single preparation of enzyme. Thus, while the values of $\Delta H^{\circ\prime}$ reported are somewhat variable (±10%), the relative values maintain. The exact value of $\Delta H^{\circ\prime}$ of Mg^{2+} depended slightly (±20%) on the specific presence of alkali metal salts (Na^+ or K^+).

The values of $\Delta H^{\circ\prime}$ for the binding of P_i and Mg^{2+} to (Ca^{2+})ATPase were very large, as previously found for the (Na^+/K^+)ATPase. These results suggest that the binding of P_i or Mg^{2+} induces a large conformational change in the enzyme molecule. These large enthalpy changes are unique characteristics of the *active* enzyme. When a sample of the enzyme was inactivated by incubation at room temperature for 24 h, mixing with Mg^{2+} at a final concentration of 15 mM produced no significant heat effect which could be associated with rapid binding. Instead, a very small positive enthalpy change followed by a very slow negative change was observed. This trace was distinctly different from all the experiments with active enzyme. Similar results had been obtained with inactivated (Na^+/K^+)ATPase (Kuriki et al., 1976).

Early experiments indicated that the binding of P_i in the presence of Mg^{2+} or the binding of Mg^{2+} in the presence of P_i reduced the apparent heat effect. Therefore, a series of studies of the binding of one ligand in the presence of the other

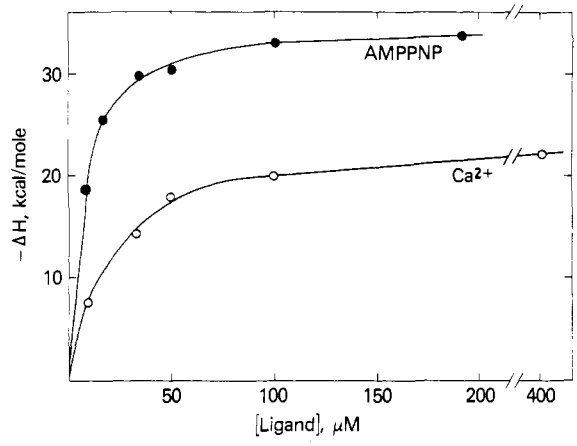


FIGURE 5: The enthalpy isotherm of Ca^{2+} binding (O) and AMPPNP binding (●) to (Ca^{2+})ATPase. The calorimetric measurements are described in the text.

were initiated. The enthalpy isotherm for the binding of P_i in the presence of 10^{-2} M Mg^{2+} is shown in Figure 3. Analysis of the data indicated that a single set of binding sites provided apparent thermodynamic values of $\Delta H^{\circ\prime} = -9$ kcal/mol and $K_d = 7 \times 10^{-3}$ M.

The binding of Mg^{2+} in the presence of 20 mM P_i produced similar results (Figure 4). $\Delta H^{\circ\prime}$ for the reaction was found to be −35 kcal/mol and $K_d = 5 \times 10^{-3}$ M. These results are analogous to the binding of P_i in the absence or presence of Mg^{2+}; the $\Delta H^{\circ\prime}$ for the binding of magnesium was reduced when P_i was present. Simultaneously, the dissociation constant of Mg^{2+} did not change significantly (the direction of change indicating improved Mg^{2+} binding). This result is consistent with a positive, cooperative interaction between the binding of the two ligands.

The enthalpy isotherm for Ca^{2+} binding in the absence of other ionic ligands is shown in Figure 5. These data, analyzed by an iterative least-squares program correcting total [Ca^{2+}] to free Ca^{2+} assuming two Ca^{2+} binding sites, yielded values of $\Delta H^{\circ\prime} = -24$ kcal/mol of protein and $K_d = 2 \times 10^{-5}$ M. The enthalpy change observed for the interaction of Ca^{2+} is specific for (Ca^{2+})ATPase. Mixing 100 μM Ca^{2+} with (Na^+/K^+)-ATPase produced an apparent heat effect of only 2 kcal/mol.

The enthalpy isotherm for the binding of the non-hydrolyzable substrate analogue App(NH)p is shown in Figure 5. Analysis of the data yielded values of $\Delta H^{\circ\prime} = -34$ kcal/mol and $K_d = 6 \times 10^{-6}$ M. The latter value is similar to the estimates of K_d for the binding of ATP. The apparent heat of Ca^{2+} or App(NH)p binding was found to be independent of the presence of the other, demonstrating that the Ca^{2+} and App(NH)p binding processes are independent.

The enthalpy isotherm for the binding of Mg^{2+} in the presence of 1.2 mM Ca^{2+} is shown in Figure 4. At this concentration of Ca^{2+}, the high-affinity Ca^{2+} binding sites are saturated and the apparent heat of Mg^{2+} binding is reduced to −35 kcal/mol. However, the sum of the heat associated with Ca^{2+} (1.2 mM) binding in the absence of Mg^{2+}, −45 kcal/mol, and the heat associated with Mg^{2+} binding at 1.2 mM Ca^{2+} is −80 kcal/mol, a value close to that obtained for binding of Mg^{2+} in the absence of Ca^{2+}. These results are consistent with a conclusion that Ca^{2+} and Mg^{2+} bind in a cooperative manner via a ligand-induced conformational change in the enzyme.

In another experiment, the binding of Mg^{2+} (at 10 mM concentration) was measured in the prescence of varying (preequilibrated) Ca^{2+} concentrations. The heat change decreased with increasing Ca^{2+} concentration approaching a limiting value of 22 kcal/mol at a Ca^{2+} concentration of 1.0 mM. The apparent dissociation constant for Ca^{2+} estimated from this experiment was approximately the same as the one obtained from the direct Ca^{2+} binding experiment (Figure 5 and Table I).

The apparent thermodynamic results for the binding experiments are summarized in Table I. In all cases, we find that the large ΔH and $T\Delta S$ values are compensated to a great extent, leading to a relatively small $\Delta G^{\circ\prime}$. The large negative ΔS values obtained with only single ligands are also suggestive of substantial conformational changes in the enzyme induced by ion binding.

Discussion

On the basis of a detailed analysis of the Mg^{2+} and P_i dependence of the phosphorylation of (Ca^{2+})ATPase by P_i and direct measurements of the enthalpy changes associated with ligand binding to the enzyme, we have concluded that this phosphorylation is related to the formation of a ternary complex of the enzyme, Mg^{2+}, and P_i. The large enthalpy and entropy changes that are known to be associated with the formation of E_2–P (Kanazawa, 1975) are in fact associated with the ligand binding to the enzyme which precedes the covalent phosphorylation step. The large, negative enthalpy and entropy changes for the binding of either Mg^{2+} or P_i and the demonstration that the magnitude of the apparent ΔH of binding one ion is reduced in the presence of the other provide strong support for the contention that the reaction is associated with a ligand-induced conformational change of the enzyme. This conclusion is consistent with the conclusion of Inesi et al. (1976) that the (Ca^{2+})ATPase exists in a temperature-dependent equilibrium between two forms.

Since the apparent heat of binding of Mg^{2+} in the presence of 1.2 mM Ca^{2+} is substantially less than in the absence of Ca^{2+}, the binding of these two ions is likely to induce a similar conformational change in the enzyme. It is to be noted, however, that while Mg^{2+} is required for the formation of phosphoenzyme from P_i, Ca^{2+} inhibits its formation or catalyzes its breakdown. Thus, phosphorylation of the enzyme from P_i and Ca^{2+} binding are mutually exclusive events. This incompatibility may play an important role in Ca^{2+} transport in the sarcoplasmic reticulum.

If P_i or Mg^{2+} induces a conformational change in ATPase which allows phosphorylation and the $\Delta H^{\circ\prime}$ is a manifestation of only the conformational change, then the $\Delta H^{\circ\prime}$ for the simultaneous binding of both ions must be greater than or equal to $\Delta H^{\circ\prime}$ for binding of either ion. For (Na^+/K^+)AT-Pase, we found that these $\Delta H^{\circ\prime}$ values were equal within experimental errors. With (Ca^{2+})ATPase, however, the $\Delta H^{\circ\prime}$ of binding P_i in the presence of Mg^{2+} or the $\Delta H^{\circ\prime}$ of binding Mg^{2+} in the presence of P_i is substantially less than $\Delta H^{\circ\prime}$ of binding of Mg^{2+} by itself. The realization that phosphorylation does not occur unless both P_i and Mg^{2+} are present leads to the conclusion that the ΔH for the covalent bond formation, $EP_iMg \rightarrow EMg\text{–}P$, may be substantially positive. Thus, the phosphorylation reaction is "high energy" in terms of enthalpy, and it is the interaction between P_i, Mg^{2+}, and the enzyme, along with the putative conformational change, which allows for activation of either the aspartate carboxyl group to serve as the phosphate acceptor or the P–O bond in the P_i to promote the departure of an OH^-.

Ca^{2+} does not appear to influence the binding of the substrate analogue, App(NH)p. This result is consistent with the observation that ATP binding is Ca^{2+} independent (Inesi & Almendares, 1968; Meissner, 1973; Neet & Green, 1977) and that ATP may have two classes of binding sites (duPont, 1977;

Taylor & Hattan, 1979). However, the binding of Mg^{2+} influences the binding of Ca^{2+} and may influence the binding of ATP through its general effect on enzyme conformation. Thus, while Ca^{2+} does not per se affect the initial substrate–enzyme interaction, Mg^{2+} may act as a coupler, probably via the phosphorylation process. deMeis & Boyer (1978) and Shigekawa & Dougherty (1978) have recently reported findings which suggest that phosphorylation changes the (Ca^{2+})ATPase from a high-affinity form to a low-affinity form. This conclusion is consistent with our thermodynamic results.

It is apparent that the Mg^{2+} dependence of the phosphorylation of (Ca^{2+})ATPase by both P_i and ATP (Panet et al., 1971; Garrahan et al., 1976; Sumida et al., 1978) reflects a conformational change necessary for phosphorylation to occur. Mg^{2+} can thus influence Ca^{2+} binding and hence play an important transport role. Mg^{2+} may stabilize the P_i-phosphorylated form of the enzyme. This form binds Ca^{2+} relatively poorly. In the absence of Mg^{2+}, the enzyme exists predominantly in a form to which Ca^{2+} binds very well. Thus, changes in $[Mg^{2+}]$ can modulate the relative amounts in the two forms, thereby controlling the Ca^{2+} transport system.

Implicit in this formulation is the hypothesis of a cyclic opening and closing of the channel which exposes the active site to varying concentrations of Ca^{2+} and Mg^{2+}. Thus, when the channel is open, Mg^{2+} is replaced by Ca^{2+}; when the channel closes, Mg^{2+} returns to the enzyme.

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