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ENTHALPY, ENTROPY AND HEAT CAPACITY CHANGES INDUCED BY BINDING OF CALCIUM IONS TO CARDIAC TROPONIN C*

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Summary Microcalorimetric titrations have been used to study the binding of Ca^{2+} to cardiac troponin C. Measurements were made both in the presence and in the absence of Mg^{2+}, and at temperatures of 5°, 15° and 25° C. Changes in enthalpy, entropy and heat capacity of troponin C associated with Ca binding have been determined. Cardiac troponin C exhibited a decrease in enthalpy and an increase in entropy associated with Ca binding. Enthalpy changes increased linearly with temperature, indicating that the Ca binding causes negative changes in the heat capacity of troponin C. These results show that the Ca binding causes a strong hydrophobic effect and a tightening of the molecular structure of cardiac troponin C.

The association of Ca^{2+} changes the molecular conformation of troponin C (TNC). Conformation changes of TNC have been extensively studied with a number of physical techniques (NMR, CD, fluorescence, etc). Calorimetric studies of skeletal TNC on Ca binding have been performed by Yamada (1), Yamada & Kometani (2) and Potter et al (3). These results have shown that Ca binding of TNC is driven by both enthalpy and entropy contributions, i.e., the binding of Ca^{2+} to TNC exhibits negative enthalpy and positive entropy changes. A thermodynamic approach to study the binding of Ca^{2+} to cardiac TNC (CTNC) has been taken by Jacobson et al. (4) using differential scanning calorimetry. They reported that the enthalpy changes for Ca binding of CTNC were positive, in contrast to those of skeletal TNC (1,2,3) and concluded that differences are due in part to inherent differences between cardiac and skeletal TNC.

In the present report calorimetric titrations were carried out in a batch microcalorimeter, which enabled us to obtain directly the enthalpy changes of

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Abbreviations: CTNC, cardiac troponin C; Pipes, piperazine-N,N'-bis(2-ethane sulfonic acid); Tris, tris(hydroxymethyl)aminomethane

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CTNC for Ca binding. Our results were inconsistent with those of Jacobson et al. (4) in that enthalpy changes were negative both in the absence and in the presence of Mg^{2+}. Instead, thermodynamic characteristics of CTNC for Ca binding were similar to those of skeletal TNC.

MATERIALS AND METHODS

Preparation of Ca- and Mg-Free CTNC. The cardiac troponin was isolated from bovine cardiac muscle by the method of Tsukui & Ebashi (5). CTNC was then prepared by successive chromatography on SP Sephadex C-50 and DEAE Sephadex A-25 in the presence of 6 M urea. CTNC was dialyzed against glass distilled water containing 2 mM $NaHCO_3$. Ca- and Mg-free CTNC was obtained by passing through a column of Dowex A-1 cherating resin (Dow Chemical Co.) (2).

Purity of CTNC was checked by electrophoresis on polyacrylamide disc gels in the presence of 0.1 % sodium dodecyl sulfate. The protein concentration was determined by the biuret reaction.

Ca and Mg concentrations were measured by atomic absorption spectrometry. The amount of Ca and Mg after the treatment with Dowex A-1 were about 0.2 mol and 0.05 mol per mol of CTNC, respectively.

Calorimetric Experiments. Calorimetric experiments were carried out in an LKB batch microcalorimeter equipped with twin gold cells. Ca^{2+} titrations were performed by adding Ca^{2+} solutions to Ca- and Mg-free CTNC from a motor driven micrometer syringe mounted on the outside of the block (2,6,7). The details of the calorimetric titrations were given in our previous paper (2).

Five ml of CTNC solution was used for an experiment. The solution was composed of 0.8 mg/ml CTNC, 0.1 MKCl and 20 mM Pipes-NaOH (pH 7.0) except for separate experiments where 20 mM Tris-HCl (pH 7.5) was used instead of Pipes buffer. The difference between the heats observed in the presence of Tris and those in Pipes was used to estimate the amount of protons released on Ca binding.

RESULTS AND DISCUSSION

Enthalpy Titrations of CTNC with Ca^{2+}. When Ca^{2+} was added to CTNC a moderate amount of heat was produced. Figure 1 shows the results of enthalpy titrations of CTNC with Ca^{2+} at 15°C. The enthalpy changes reached plateaus at about 2 mol Ca^{2+} per mol of CTNC in the absence as well as in the presence of Mg^{2+}. At 15°C plateau values were -18.2 and -24.5 kJ/mol CTNC in the absence and in the presence of Mg^{2+}, respectively.

Results shown in Fig.1 should be compared with those of Jacobson et al. (4) who reported positive enthalpy changes for Ca binding of CTNC at 25° and 37°C. Jacobson et al. (4) studied under higher KCl and $MgCl_2$ concentrations, therefore the difference between the present results and the results of Jacobson et al. (4) could be attributed to the difference in experimental conditions. However, enthalpy changes at higher KCl and $MgCl_2$ concentrations were also negative though the plateau value was lower than that at lower KCl

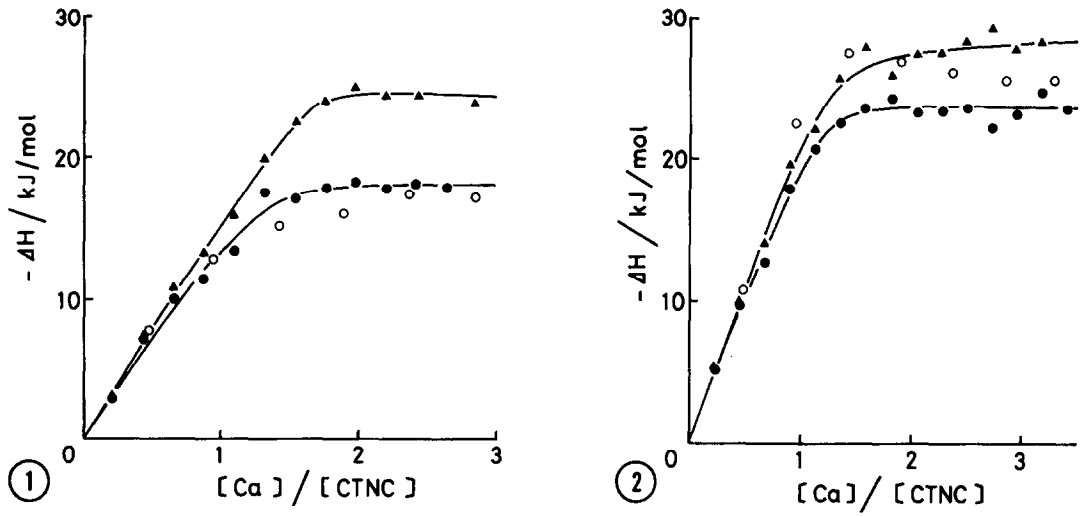

Figure 1. Enthalpy titrations of CTNC with Ca at pH 7.0 and at 15°C. Heat observed per mole of CTNC is plotted against the amount of Ca added per mole of CTNC. Closed circles, the titration in the absence of Mg^{2+}; closed triangles, the titration in the presence of 1 mM Mg^{2+}; open circles, the titration under higher salt concentrations (0.5 M KCl and 10 mM $MgCl_2$).

Figure 2. Enthalpy titrations of CTNC with Ca at pH 7.0 and at 25°C. Closed circles, the titration in the absence of Mg^{2+}; closed triangles, the titration in the presence of 1 mM Mg^{2+}; open circles, the titration in the absence of Mg^{2+} where 20 mM Tris-HCl (pH 7.5) was used instead of Pipes buffer.

and $MgCl_2$ concentrations (Fig.1). The present results clearly show that Ca binding to CTNC is an exothermic process.

Titrations were also made at 25° and 5°C. Figure 2 shows the results of enthalpy titration at 25°C. The plateau values at 25°C were -23.6 and -25.2 kJ/mol in the absence and in the presence of Mg^{2+}, respectively. At 5°C the enthalpy changes were also negative and titration curves were similar to those in Figs. 1 & 2. Plateau values at 5°C were -6.0 and -16.2 kJ/mol in the absence and in the presence of Mg^{2+}, respectively.

Figure 2 also shows the difference between the results of enthalpy titration obtained in the presence of Tris and those in the presence of Pipes, at 25°C. The plateau values in Tris (ΔH_{Tris}) were larger than those in Pipes (ΔH_{pipes}). Since the protonation heat of Tris and Pipes at 25°C are -47.2 and -11.4 kJ/mol (8,9), respectively, the difference, 2 kJ/mol, between ΔH_{Tris} and ΔH_{pipes} should correspond to 0.056 mol of protons released per mol of CTNC associated with Ca binding. This amount of protons should have produced heat

Table 1. Binding parameters of CTNC on Ca binding

	Mg free			1 mM Mg		
temp.	n	log K	$\frac{\Delta H}{kJ/mol}$	n	log K	$\frac{\Delta H}{kJ/mol}$
5°	1.3	7.2	-4.99	1.5	6.6	-13.5
15°	1.6	7	-12.41	1.8	6.8	-15.04
25°	1.4	6.6	-19.25	1.5	5.8	-19.92

of 0.64 kJ/mol at 25°C by associating with Pipes, which must be subtracted from the observed heat. However, the protonation heat is small enough compared to the observed heat, therefore no allowance was made for the protonation heat.

Binding Parameters and Thermodynamic Functions of CTNC on Ca Binding.

A multiple regression analysis was used to estimate values of the binding parameters n, log K and ΔH. Details of analysis were given in our previous paper (2). An assumption made was that CTNC has n identical and independent sites, whose binding constant and enthalpy change associated with Ca binding are K and ΔH, respectively. The binding parameters thus obtained are given in Table 1.

It has been reported that CTNC has two high affinity and one low affinity Ca sites (10,11,12). Others have indicated only two Ca sites (13,14). Our results show that only two Ca sites are seen in the calorimetry. The binding constants of these two sites obtained in the present study are in agreement with those for the two high affinity Ca sites reported by Horloyde et al. (10) and Leavis and Craft (11). If CTNC has three Ca sites, the Ca binding to the 3rd site would be thermally neutral.

The standard free energy changes (ΔG°) are calculated from the values of log K in Table 1 according to the equation $\Delta G° = -2.3RT\log k$, where R is gas constant and T the temperature. The changes in the entropy (ΔS°) were then calculated from ΔG° and ΔH. In the absence of Mg^{2+}, ΔS° is the entropy change for reaction CTNC + Ca $\rightleftarrows$ CTNC-Ca, i.e., $\Delta S° = S°(CTNC\text{-}Ca) - S°(CTNC) - S°(Ca^{2+})$ Hence the entropy change (ΔS(CTNC)) of CTNC molecule associated with Ca binding is obtained by the equation $\Delta S(CTNC) \equiv S°(CTNC\text{-}Ca) - S°(CTNC) = \Delta S° + S°(Ca^{2+})$.

Table 2. Thermodynamic functions of CTNC on Ca binding at 15°C

	$\Delta G°$ kJ/mol	$\Delta S°$ J/mol/deg	ΔS(CTNC) J/mol/deg	$\Delta G°$ J/mol/deg	ΔCp(CTNC) J/mol/deg
Mg free	-38.5	90.7	25.74	-713	-871.8
1 mM Mg	-37.4	77.8	140.5	-321	-375.3

In the presence of Mg^{2+}, bound Mg^{2+} is replaced by Ca^{2+} (10,11) and the expression for ΔS(CTNC) would be ΔS(CTNC) = $S°$(CTNC-Ca) - $S°$(CTNC-Mg) = $\Delta S°$ + $S°(Ca^{2+})$ - $S°(Mg^{2+})$. The values of the standard aqueous entropy $S°(Ca^{2+})$ and $S°(Mg^{2+})$ are -64.96 and -127.66 J/mol/K (2,15,16), respectively. $\Delta G°$, $\Delta S°$ and ΔS(CTNC) at 15°C calculated in this way are given in Table 2.

The heat capacity changes ($\Delta Cp°$) are also tabulated in Table 2. The values of $\Delta Cp°$ were obtained from the temperature dependence of ΔH (2). The quantity ΔCp(CTNC) in Table 2 is the heat capacity change of CTNC molecule associated with Ca binding and its calculation is similar to those of ΔS(CTNC). The values used for $Cp°(Ca^{2+})$ and $Cp°(Mg^{2+})$ are -158.84 and -104.5 J/mol/K (2, 15,17), respectively. The results in Table 1 & 2 show that the Ca binding to CTNC is driven both by enthalpy and by entropy. The binding of Ca^{2+} gives rise to a decrease in heat capacity and an increase in entropy of CTNC molecule.

Studies by CD spectrum (11,18) have shown that Ca binding causes an increase in the helix content of CTNC, resulting in a decrease in internal degrees of freedom of CTNC molecule. A decrease in internal degrees of freedom should cause a decrease both in heat capacity and in entropy of CTNC molecule, whereas our results show a decrease in heat capacity and an increase in entropy of CTNC associated with the binding of Ca^{2+}. Hence, the Ca binding to CTNC involves other effects that cause the overall entropy change to be positive. One of the most important effects among them should be the hydrophobic effect. Thus the binding of Ca^{2+} causes a decrease in the extent of interaction between water and the hydrophobic groups of CTNC, resulting in a decrease in heat capacity and an increase in entropy of CTNC. This effect could be associated with an increase in the helix content of CTNC.

These characteristic features of Ca binding to CTNC are similar to those of skeletal TNC (2). However, CTNC differs from skeletal TNC in the characteristics seen in the absence of Mg^{2+}. Our previous studies (2) have shown that, in the absence of Mg^{2+}, two high affinity Ca sites in skeletal TNC differ from each other in their binding constant for Ca^{2+} and in thermodynamic functions for Ca binding, whereas the present study shows no such distinction between two high affinity sites in CTNC. Thus two high affinity sites in CTNC are identical both in the absence and in the presence of Mg^{2+}.

It has been suggested that the low affinity Ca site is more important in the regulation of contraction of cardiac muscle (10) as well as that of skeletal muscle (2,19). However, the enthalpy change of Ca binding to the low affinity Ca site, if present, was undetectably small in our calorimetry. This could be related to the fact that the amount of the activation heat in cardiac muscle is only 20% of that in skeletal muscle (20).

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