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NUCLEOTIDE-INDUCED CHANGES IN THE HEAT CAPACITY OF BEEF CARDIAC MYOSIN

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The heat of reaction of ATP with beef cardiac myosin has been determined in a flow microcalorimeter at two different temperatures. The reaction heat obtained by extrapolation to infinite flow rate is interpreted to be the heat of formation of the steady-state set of myosin-nucleotide complexes. The large temperature dependence of this heat, an apparent change in heat capacity, could be caused by an isomerization between two myosin conformations. The enthalpies and heat capacities of binding of ADP, AMP, and pyrophosphate have also been measured and are discussed in terms of this model.

1. Introduction

Muscle fibers contain arrays of two types of interdigitating protein filaments consisting of polymers of either myosin or actin which touch each other by means of extensions of the myosin molecule called cross-bridges. The cross-bridges contain active sites where ATP is hydrolyzed to supply the driving energy for muscle contraction.

One of the goals of research on the biochemistry of muscle contraction has been to correlate the steps of the kinetic mechanism of the ATPase activity of myosin [1] with the mechanical cycle of cross-bridge movement based on X-ray and electron micrographic studies of intact muscle fibers [2]. Stopped-flow kinetic studies of the intermediate steps of the Mg^{2+}-ATPase activity of a proteolytic fragment of rabbit skeletal myosin called subfragment-one (S-1) [3] have provided evidence for one or more temperature-dependent isomerizations that involve changes in the protein fluorescence. These isomerizations of S-1 may be related to cross-bridge movement in muscle fibers, but understanding has been hampered by a lack of physical chemical data about them.

Cardiac muscle fibers contain the same basic filament structure as skeletal muscle fibers but the myosin consists of an isozyme whose maximum rate of ATP hydrolysis is substantially lower [4]. The kinetic mechanism, however, appears to be qualitatively the same, differing only in the rate constants of some of the steps [5].

Recently, Kodama [6,7] and Kodama and Woledge [8] have presented calorimetric evidence that there are large changes in heat capacity associated with formation of S-1-nucleotide complexes. In this report we present similar observations for intact cardiac myosin obtained with a different type of calorimetric instrumentation, and we point out that these large heat capacity effects could represent a reaction heat contribution from isomerization between two myosin conformations.

2. Materials and methods

Myosin from beef hearts was prepared as described previously [9]. Myosin concentrations

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(6–10 mg/ml) were determined from the absorbance at 280 nm corrected for light scattering and by use of an extinction coefficient $E_{280} = 5.6$ [10] and $M_r = 470\,000$. Since myosin is insoluble in low salt solution, all experiments were done in 0.5 M KCl.

The enthalpy data in this paper were obtained with an LKB flow microcalorimeter [11] as described previously [12]. The use of this calorimeter to obtain kinetic data has been described by Johnson and Biltonen [13] and has been extended by Fisher et al. [14] to multistep reactions.

In kinetic experiments described in this paper, solutions of myosin and ATP at fixed concentrations are pumped into the calorimeter and mixed inside. Heats of dilution of myosin and ATP, determined in separate control experiments, are subtracted from the observed heat of mixing. This procedure is repeated at various flow rates so that in each experiment the solutions spend a different length of time in the calorimeter after mixing. The corrected heat of mixing reflects the heat of formation of the myosin-product complexes present in the steady-state mixture, and the heat of hydrolysis of that portion of the ATP hydrolyzed during the residence time in the calorimeter due to enzyme turnover. Extrapolation of the observed heat to infinitely fast flow rate gives the true heat of binding of ATP to myosin plus the heat of conversion of the bound ATP to the set of bound hydrolysis product[illegible]h is present at steady-state.

This use of [illegible]w microcalorimeter to obtain kinetic informa[illegible]s more straightforward than the use of a batch device [6], because the batch calorimetric signal must be deconvoluted into a fast and slow component. This process can be relatively complex, as discussed by Randzio and Suurkuusk [15].

3. Results

Fig. 1 shows the heat produced when excess ATP is mixed with beef cardiac myosin in a flow calorimeter at various flow rates. The concentrations of ATP and myosin are held constant. The ordinate of fig. 1 is heat change (kcal) per mol myosin active site.

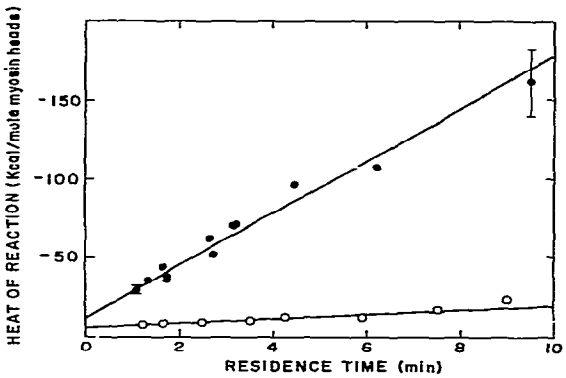

Fig. 1. Heat of reaction of ATP with bovine cardiac myosin as a function of residence time in a flow calorimeter. Experiments were carried out at 25 °C (●) and 2 °C (○) in 0.02 M Hepes (pH 7.5), 10 mM $MgCl_2$, 0.5 M KCl with 1 mM ATP. Lines are the result of linear regression analysis in which points were weighted according to the error in the experimental signal. Fitted parameter values with standard deviations for the intercepts (heats of formation of the set of steady-state intermediates) are -11.5 ± 0.4 kcal/mol at 25 °C and -6.04 ± 0.09 kcal/mol at 2 °C. These values for slopes (steady-state turnover number) are 0.029 ± 0.0004 s^{-1} at 25 °C and 0.0024 ± 0.00006 s^{-1} at 2 °C. The bars indicate the standard deviation of the 25 °C data at the highest and lowest flow rates, calculated from the regression analysis.

The apparent heat of reaction of ATP with cardiac myosin (fig. 1) can be experimentally divided into two parts: (1) heat from fast reactions which are completely finished during the residence time in the mixing cell and will therefore be independent of flow rate and (2) heat from slow reactions which are only partially completed during the residence time and will therefore be dependent on flow rate.

3.1. Slow reactions

It has been demonstrated [13] that the heat produced by a slow reaction in this calorimeter accurately reflects the amount of product formed during the residence time in the calorimeter and can be used to determine the kinetic parameters of the reaction. Therefore, we interpret the slope of the straight line through the data in fig. 1 to represent the heat per unit time of slow reactions

Table 1

Mg^{2+}-ATPase activity of bovine cardiac myosin

Method	Temperature (°C)	Activity (nmol P_i/mg per min)	Reference
Calorimetric [a]	25	7.28	This work
P_i analysis [b]	25	3.67	This work
Spectrophotometric	25	4.85	10
Calorimetric [a]	2	0.60	This work
P_i analysis [b]	0	0.46	This work

[a] Data of fig. 1. Conditions: 20 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 0.5 M KCl, 1 mM ATP, 5 mg/ml myosin. The slope of the line in fig. 1 was converted to steady-state turnover number by dividing by the effective volume of the calorimeter (0.4 ml) and by the heat of hydrolysis of ATP under these conditions (−9.8 kcal/mol). The heat of hydrolysis of ATP consists of the intrinsic heat of hydrolysis (−4.8 kcal/mol [6]) and the heat of neutralization of 1 mol H^+ by Hepes buffer (−5.0 kcal/mol [16]).

[b] P_i release determined by the method of Fiske and Subbarow [17] after 5 min (25°C) or 81 min (0°C) in 20 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 0.5 M KCl, 5 mM ATP, 1–2 mg/ml myosin. At 25°C about 10% of the activity is lost after 10 min.

initiated by the mixing of ATP and cardiac myosin.

The primary slow reaction is the steady-state hydrolysis of ATP by myosin. As shown in table 1, the calorimetrically measured steady-state rate is somewhat larger than the steady-state rate measured by analysis of phosphate release. At 25°C it is about 50% larger and at 2°C about 25% larger. There is a plausible explanation for this discrepancy: cardiac myosin appears to undergo a slow aggregation and disaggregation reaction which is coupled to ATP hydrolysis [10,18,19]. The scatter in the data at 25°C in fig. 1 can be largely attributed to this problem because upper data points were obtained using a 1 : 3 dilution and the lower data points using a 1 : 2 dilution. In any case, the best straight line through the 1 : 3 dilution data points and the 1 : 2 dilution data points gives the same intercept within error on the ordinate, indicating that the heat at infinite flow rate does not contain a significant contribution from this effect.

3.2. Fast reactions

The value for the reaction heat at infinite flow rate is for formation of the set of bound products present in the steady-state mixture.

The steady-state set of myosin-product complexes can be defined by the kinetic mechanism proposed by Bagshaw and Trentham [3] and others for skeletal myosin S-1 at 25°C: ATP is quickly bound (step 1), an isomerization occurs increasing the fluorescence (step 2), and ATP is split into ADP and P_i accompanied by a further increase in fluorescence (step 3).

$$M + ATP \overset{1}{\rightleftharpoons} M\,ATP \overset{2}{\rightleftharpoons} M^*ATP \overset{3}{\rightleftharpoons} M^{**}ADP \cdot P_i$$

$$\underset{\text{slow}}{\overset{4}{\rightleftharpoons}} M^*ADP + P_i \overset{5}{\rightleftharpoons} M \cdot ADP \overset{6}{\rightleftharpoons} M + ADP$$

The rate-limiting step (4) is an isomerization with a decrease in the protein fluorescence, accompanied by the release of P_i and a proton. Because the release of products is slow, the steady-state mixture during myosin catalysis consists almost entirely of the M*ATP and M**ADP · P_i complexes. The situation at low temperature is less clear-cut: the rate of release of ADP (steps 5 and 6) becomes sufficiently slow that a significant amount of the steady-state complexes lack bound P_i.

Taylor and Weeds [10] have shown that this kinetic mechanism is followed by beef cardiac myosin, except the isomerization steps are slower than with skeletal myosin.

The extrapolations of the data of fig. 1 to infinite flow rate show that the enthalpy of formation of myosin-nucleotide complexes present at steady-state changes from −11.5 to −6.0 kcal when the temperature is lowered from 25 to 2°C. The corresponding heat capacity is too large to be accounted for by a change in the binding of protons or phosphate ion. The above heat capacity values are nearly identical to the values reported by Kodama [6] for the formation of skeletal myosin S-1-product complexes.

3.3. Binding reactions

Table 2 summarizes thermal titrations of cardiac myosin with ADP, AMP, and pyrophosphate and compares them to the ATP results of fig. 1. The thermodynamic parameters show similarities and differences; the most striking pattern is that ATP, ADP and pyrophosphate exhibit large heat capaci-

Table 2

Thermodynamic quantities for beef cardiac myosin-ligand interactions at 25 °C [a]

Ligand	$\Delta H°$ (kcal/mol)	ΔC_p (cal/K per mol)	ΔH^+/site [f]
PP_i	−6	−140	0.07
AMP	−5	−15	−0.33
ADP	−14.5	−270 (−178) [d]	−0.4
ATP [b]	−11.5	−240 (−365) [e]	−0.23 [e]

[a] Experiments (except with ATP) were carried out in 0.02 M Tris-HCl solution (pH 7.5) with 0.5 M KCl and excess ligand. Enthalpy values were calculated per mol ligand assuming 2 mol ligand bound per mol myosin and were not corrected for the heat of ionization of the buffer. Heat capacities (except with ATP) were determined by comparing enthalpies at 25 and 10 °C. The change in protons bound per site (ΔH^+/site = uptake) was determined by comparing binding enthalpies in two different buffers (Tris and Hepes or Pipes).

[b] Values are for the formation of the set of steady-state intermediates using data from fig. 1.

[c] Value for rabbit skeletal S-1 from ref. 3.

[d] Value for rabbit skeletal S-1 from ref. 7 for 25–10 °C interval corrected for heat of proton ionization.

[e] Value for rabbit skeletal S-1 from ref. 6 for 23–4 °C interval uncorrected for heat of ionization.

[f] The heat of ionization of Tris is +11.34 kcal/mol at 25 °C and +11.7 kcal at 10 °C [16]. See ref. 20 for a discussion of the contribution of H^+ uptake and release to the apparent ΔC_p of binding.

ties of binding while AMP does not. This correlates with their abilities to dissociate actin and myosin [21].

4. Discussion

Sturtevant [22] and Eftink and Biltonen [20,23] have recently attempted to account for the large heat capacity changes seen with some [24], but not all ligand-binding reactions with proteins [25]. The possible sources of this effect can be divided into two broad categories: (1) effects directly related to the process of removing the ligand from water to the binding site and (2) ligand-induced shifts in the state of the protein molecule.

Eftink et al. [23,26] have made a careful study of the binding of 3′-CMP (product) and cyclic 2′,3′-CMP (cCMP) (substrate) to ribonuclease; which are nucleotide-binding reactions exhibiting somewhat smaller changes in heat capacity than cardiac myosin with ATP or ADP. They estimate the contribution to the heat capacity of the direct effects of 3′-CMP or cCMP binding account for a third or less of the observed ΔC_p. They point out that pyrimidine nucleotides cannot be characterized as hydrophobic molecules and that many of the heat capacity effects associated directly with binding 3-CMP or cCMP are of different sign and tend to cancel. They assign most of the observed heat capacity of binding to the second category: an induced change in the heat capacity of the protein molecule as a whole.

Eftink et al. [23] go on to describe two fundamentally different sources of ligand-induced heat capacity changes in the protein molecule: (1) a change in the energy distribution of the vibrational modes of a single protein conformation upon ligand binding, which is a contribution emphasized by Sturtevant [22], and (2) a poised, temperature-dependent equilibrium between two discrete conformations of the protein, one of which binds ligand tighter than the other.

A particularly clear example of how a ligand-induced conformational change can explain a large ΔC_p of binding has been reported by Fisher et al. [27]. To interpret their data they use a model in which the binding of NADPH to glutamate dehydrogenase is coupled to an enzyme isomerization with $K_{eq} = 1$ at 43.6 °C and $\Delta H = -20$ kcal/mol. As the temperature is raised from 5 to 35 °C, the ΔH of ligand binding becomes more exothermic (+4.5 to −1.5 kcal/mol), which produces an apparent ΔC_p of binding of over −400 cal/K per mol at 35 °C. They point out that isomerization of the free protein will always produce a negative apparent heat capacity change and the magnitude of ΔC_p will be proportional to the square of the ΔH of the isomerization.

The nucleotide-binding data reported in this paper using bovine cardiac myosin and those obtained by Kodama [6,7] using rabbit skeletal S-1 can be explained most simply and plausibly by a similar ligand-induced conformational change. This, of course, does not prove that a conformational change actually occurs when ATP or ADP bind to myosin, but in analogy with the binding of 3′CMP and cCMP to ribonuclease described by

Eftink et al. [23,26], the ΔC_p of binding of ATP and ADP to myosin is too large to be accounted for simply by local interaction at the binding site. This is reinforced by the small ΔC_p of binding that we have measured for AMP binding to myosin. In terms of this model, AMP binding is not linked to the conformational change and reflects only the intrinsic binding of a nucleotide.

This model for myosin also agrees with one proposed by Shriver and Sykes [28]. Their NMR data point to the existence of two forms of S-1-ADP complex, one predominating at 25°C and the other favored at 4°C, with an apparent ΔH of interconversion between the two forms of 30 kcal/mol. The theoretical calculations of Eftink and Biltonen [20,23] indicate that this is large enough to produce the experimentally observed ΔC_p values of binding.

Biosca et al. [29] have used a similar model to interpret the kinetics of binding of ATP to S-1. They attribute a discontinuity in the Arrhenius plot of their data (i.e., an apparent heat capacity of activation) to a 'phase transition' in the S-1 molecule.

If this model is correct, measurement of binding enthalpies and heat capacities would serve as a method for monitoring the status of the conformation of the myosin molecule under various sets of conditions.

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