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Nucleotide-induced changes in muscle fibres studied by DSC and TMDSC

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Abstract

Differential scanning calorimetry (DSC) was used in conventional and temperature-modulated mode to study the energetics of myosin in skeletal muscle fibres in different states of the actomyosin ATPase cycle. Psoas muscle fibres from rabbit were used in the experiments with and without the presence of nucleotides (ATP, ADP, AMP–PNP) and ATP or ADP + orthovanadate.

In the complex DSC pattern, the higher transition referred to the head region of myosin. The enthalpy of the thermal unfolding depended on the nucleotides, the conversion from a strongly attached state of myosin to actin to a weakly binding state was accompanied with an increase of the transition temperature which was due to the change of the affinity of nucleotide binding to myosin. This was more pronounced in TMDSC mode, indicating that the strong-binding state and rigor state differ energetically from each other. The different transition temperatures indicated alterations in the internal microstructure of myosin head region. The monoton decreasing TMDSC heat capacities show that the C_p of biological samples should not be temperature independent.

Keywords: DSC; Temperature-modulated calorimetry; Skeletal muscle contraction; Nucleotides

1. Introduction

Muscle is a chemo-mechanical machine that converts chemical energy into work and heat. The energy source is the myosin-catalysed ATP hydrolysis; the rate is enhanced in the presence of actin [1,2]. In the presence of MgATP, the energy released from hydrolysis produces conformational changes in myosin [3] and/or in actin [4,5], which can be manifested as an internal rotation of myosin head while bounds to actin. The force generation is performed by cyclic interaction of myosin with ATP and actin, and at least six intermediates are proposed for actomyosin ATPase in solution [6]. The better understanding of the interrelation between the chemical events observed in protein solutions and the mechanical activity on cellular level requires experiments on supramolecular complexes, where stabilising forces may modulate the hydrolysis process. We have extended the experiments to study the fibre system prepared from psoas muscle of rabbit in rigor, strongly binding and weakly binding states of myosin to actin where P_i was substituted by the phosphate analogue orthovanadate [7,8].

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Studies using different techniques indicated that the nucleotide-binding pocket did not experience large conformational changes during the hydrolysis cycle [8–10]. However, the small nucleotide-induced conformational changes in the motor domain should be converted into larger movement. Data agree that while the structure of the motor domain remains similar to rigor, the regulatory domain swings about a point in the distal end of the motor domain [11]. The changes in the 50 kDa domain might affect the segment of the 20 kDa domain that contains the essential sulfhydryl groups.

In this report, we studied the effect of MgADP and MgATP–orthovanadate in the ATPase cycle with conventional and temperature-modulated DSC measurements that report domain stability and interactions.

2. Materials and methods

2.1. Materials

Potassium chloride (KCl), magnesium chloride ($MgCl_2$), ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid (EGTA), histidine–HCl, glycerol, adenosine 5′-diphosphate (ADP), adenosine 5′-triphosphate (ATP) and orthovanadate (Na_3VO_4) were obtained from Sigma (Germany).

2.2. Fibre preparation

Glycerol-extracted muscle fibre bundles were prepared from rabbit psoas muscle. Small stripes of muscle fibres were stored after osmotic shocks in 50% v/v glycerol and buffer solution (80 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 25 mM Tris–HCl, pH 7.0, rigor buffer) at −18°C up to 1 month. Fibre bundles of glycerinated muscle were washed for 60 min in rigor buffer to remove glycerol, and then transferred to fresh buffer. This state models the rigor state of the muscle (AM, where M denotes myosin and A stands for actin). 5 mM MgADP was added to the rigor solution to simulate the strongly binding state of myosin for actin which may correspond to the AM–ADP state. In experiments involving MgADP, the activity of adenylate kinase was inhibited by addition of 50 μM diadenosine pentaphosphate. The other analogue of intermediates in the ATPase pathway was formed by ADP or ATP + orthovanadate (abbreviated as V_i), which together bind stoichiometrically at the active site of myosin to form a stable complex AM–ADP–V_i. The muscle fibres were stored in solution containing 80 mM K-propionate, 5 mM $MgCl_2$, 5 mM ADP or 5 mM MgATP + 5 mM V_i, 1 mM EGTA in 25 mM Tris–HCl buffer, pH 7.0 for 15 min at 0°C before measurements.

2.3. Calorimetric measurements

The thermal unfolding of myosin in fibres was monitored by a SETARAM Micro DSC-II calorimeter (SETARAM, France). All experiments were done between 0 and 100°C with a scanning rate of 0.3°C/min. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μl sample volume in average. Rigor buffer was used as reference sample. The sample and reference vessels were equilibrated with a precision of ±0.1 mg. There was no need to do any correction from the point of view of heat capacity between the sample and reference vessels. The samples were irreversibly denaturated during each cycle. The modulated DSC measurements were performed by series with temperature jump of 0.5°C under 900 s followed by 900 s isotherms.

2.4. Evaluation of DSC scans

The repeated scan of denaturated sample was used as baseline reference which was subtracted from the original DSC scan. After SETARAM ASCII conversion, the deconvolution was performed with PeakFit 4.0 program from SPSS Corporation. Gaussian peak functions were used to approximate the unfolding of the structural units of myosin. Calorimetric enthalpy was calculated from the area under the heat absorption curves using two-point setting SETARAM peak integration. Fast Fourier transformation developed in C. Schick's laboratory was applied to evaluate the modulated scans.

3. Results

3.1. DSC measurements

The unfolding of proteins in muscle fibres by thermal excitation is a complex process and depends

on the state of the actomyosin complex. The experimental data suggest at least four transitions in the temperature range examined. The minor transition at $T_{m1} = 18^{\circ}C$ is not shown in the figures; it might be attributed to the interaction of LC-2 light chain with the long α-helical part of the myosin head [12]. This transition could be evaluated as the sign of an inter-domain communication.

The starting point of the evaluation was that the main transition between 45 and 70°C is the superposition of endotherms that are believed to correlate mainly with T_m of the larger domains of myosin and a smaller contribution from thin filaments. On isolated myosin, three endotherms were reported, therefore in the deconvolution procedure, three transitions were assumed.

In the absence of nucleotides, three transitions could be decomposed from the DSC scan with $T_{m1} = 54.05^{\circ}C$, $T_{m2} = 58.36^{\circ}C$ and $T_{m3} = 62.29^{\circ}C$ melting temperatures (Fig. 1, bottom). In the presence of MgADP (Fig. 1, middle), there is an increase in the peak of transition temperature 53.5°C, which could be

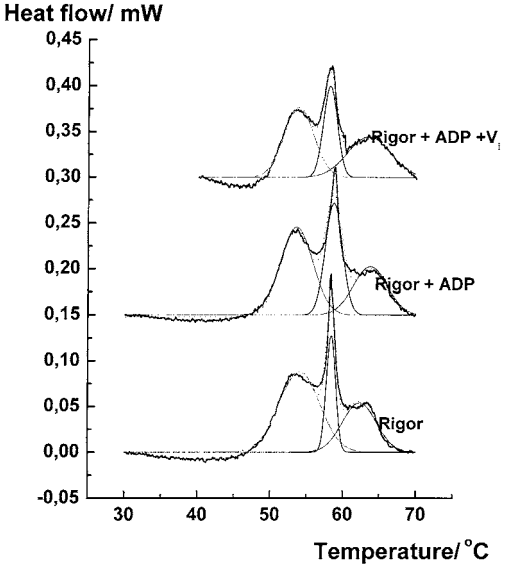

Fig. 1. DSC patterns of muscle fibre system in rigor — bottom: no nucleotide is present; middle: AM–ADP or strong binding state; upper: AM–ADP–V_i or weak binding state. Symbols — raw line: experimental DSC scan without filtering; dotted line: sum of the deconvoluted curves. The deconvolution procedure was performed using PeakFit 4.0 program from SPSS Corporation. In order to describe the single transitions, Gaussian functions were used.

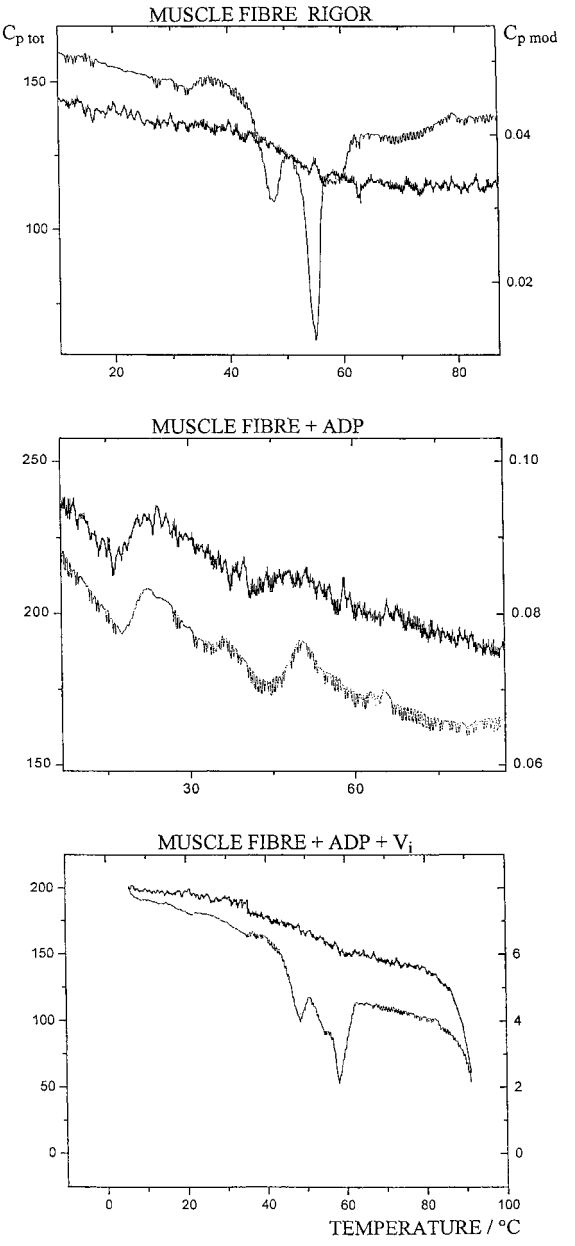

Fig. 2. TMDSC scans of different states of muscle contraction in rabbit psoas fibres — left axis: $C_{p,total}$; right axis: $C_{p,modulated}$. Conventional DSC scans plotted in grey, modulated curves in black.

the energetic consequence of the internal rearrangement of myosin structure [11,13].

When 5 mM MgADP was added in the presence of 5 mM V_i, the separation of the first two peaks is less

Table 1
DSC parameters of melting of contractile proteins in fibre system[a]

Muscle state	Transition temperature (°C)			Excess enthalpy (J/g)		
	T_{m1}	T_{m2}	T_{m3}	H_1	H_2	H_3
Rigor	54.05	58.36	62.29	54.08	18.06	27.85
ADP	53.49	58.61	63.64	44.51	29.96	25.51
ADP–V_i	53.98	58.08	63.78	40.94	25.14	33.91

[a] Glycerinated muscle fibres isolated from psoas muscle of rabbit were measured in different intermediate state of the ATP hydrolysis cycle. The states were rigor state, strong-binding (ADP) state and weak-binding (ADP–V_i) state.

pronounced (Fig. 1, upper). It is obvious that addition of nucleotides produces conformational changes in the multisubunit structure of myosin. The enthalpy of the thermal unfolding depends on the nucleotides, the conversion from a strongly attached state of myosin for actin to a weakly binding state is accompanied with an increase of the transition enthalpy of the third melting peak which might relate to the change of the affinity of nucleotide binding to myosin (Table 1).

3.2. TMDSC measurements

The total C_p in each case of simulated muscle contraction showed practically the same result as the conventional DSC scans. The very surprising new finding was that the modulated C_p instead of sharp transitions revealed continuous decrease in the function of temperature in the main melting range (Fig. 2). This supports the proposal of Chun [14,15] that during the unfolding of proteins in the calculation of different thermodynamic functions, the C_p cannot be handled as constant, temperature independent variable.

4. Discussion

The structure of actomyosin complex is well known in the absence of ATP [3], but the structural changes which are followed during tension development is less understood. It was proposed that this process involves the transition of the myosin head from a ''weak'' to a ''strong'' binding state to actin [2,16–21]. The constrain generated by the filament association and protein–protein interaction increases the rigidity in the supramolecular structure, and this stabilises the system. The structure formation alters the dynamical and energetical behaviour of contractile proteins, the consequence of that is the shift of the melting points 39, 47 and 51°C, measured in solution on myosin, to higher temperatures in rigor (Fig. 1). This is an evidence that particular regions of myosin are subjected to stabilising forces leading to alteration of the transition temperatures (Table 1).

Earlier studies on myosin suggested that the endotherms were correlated with the T_m of the principal domains of the myosin molecule, and concluded that the head region (S-1) has a distinct transition around $T_m = 52.5$°C in 0.1 M KCl (pH 7.0), whereas the long tail (LMM) showed two endothermic transitions ($T_m = 45$ and 56°C) at high salt concentration [21]. Precise DSC measurements allowed the deconvolution of the melting curve on S-1 and three transitions were obtained [22]. The unfolding around 47°C may refer to the catalytic domain in S-1 [12,23] which is the most labile part of S-1, while the transitions at higher temperature could be assigned to the unfolding of the long α-helical part of the myosin head [1,24,25]. Nucleotides (ADP, AMP.PNP) and nucleotides+ orthovanadate affected differently the thermal transitions of the different domains [22–26]. The melting of actin appeared at 63°C [27].

The results cited above suggest that a clear and unique assignment of the thermal transitions to the components of muscle fibres is not easily possible. The only way to obtain information about the structural domains of myosin and their thermal properties is the simulation of the different intermediate states of the ATP hydrolysis under controlled conditions. As a starting point, we assumed that at first approximation, the contributions from the LMM and actin filaments are the same in the intermediate states, independently of the state of myosin heads. In the presence of 5 mM MgADP, a significant decrease in the enthalpy of the transition temperature 54°C — it is shifted to 53.49°C as well — was calculated which might be an energetic consequence of the internal rearrangement of myosin structure (Fig. 1). The binding of ADP can induce loosening of the association between the myosin domains and/or a decreased interaction between the two myosin heads [22,28]. When 5 mM MgADP+ 5 mM V_i were added to the muscle sample, the first

two transitions were less separated and pronounced (Fig. 1).

Recent data suggest that MgADP induces an internal change in the multisubunit structure of S-1 which were served by spectroscopic [13,29] and electrical birefringence [30] techniques. In the presence of MgADP, the myosin heads remain attached to actin, as could be seen from the ST EPR spectra, the spectral parameters L''/L were the same in rigor and in ADP state in the limits of the experimental error. In contrast, large difference in the static order of the attached labels was detected in the conventional EPR spectra, evidencing that the binding of MgADP allowed the rotation of the segment that holds the label from one conformationally stable state into another one, and this is accompanied by a subtle rotation of the entire head [29,31–34].

Very likely, the myosin head has only one stereospecific orientation with that it binds strongly to actin, but with different internal structure depending on bound nucleotide. It was already reported that nucleotide binding influenced the region containing the essential sulfhydryls [33,35,36].

It is obvious from our data that the strongly binding state and rigor state differ energetically from each other Table 1. Saturation of myosin with MgADP leads to stabilisation of the head regions [22], which is reflected in the increased excess enthalpy H_2 at $T_{m2} = 58.6^{\circ}C$. In contrast to this enthalpy change, there is a decrease of excess enthalpy H_1 at $T_{m1} = 53.5^{\circ}C$. Very likely, the binding of ADP results in a decreased interaction between the two myosin heads. This interpretation implies a small global conformational change of the myosin head following ADP binding. The active site pocket in ADP state has nearly the same closed conformation as in rigor, and this results in a strong interaction between myosin and actin. The increase of excess enthalpy H_2 observed in strongly and weakly binding states could be a sign of the ''stretched state'' of the myosin heads during force generation. It seems the middle part of the decomposed DSC scan (Table 1) may refer to the nucleotide binding domain which undergoes small conformational change in different states, as seen in the EPR spectra. However, the transition temperature exhibits only subtle change, evidencing that the global structure in rigor and in strong-binding state does not differ significantly.

The third component of the main transition might refer partly to the rod part of myosin [37,38] and the melting of actin [39]; but its broadening and small shifting towards higher temperature and yielding a greater transition enthalpy in weakly binding state (AM–ADP–V_i state) could be the sign that the environment of the nucleotide pocket becomes more packed in the presence of orthovanadate. It was shown by fluorescence measurements that the distance between εADP and SH1 is shorter in S-1–εADP–V_i complex, but this distance is unperturbed in acto-S-1–εADP complex [40]. Comparison with various nucleotide-bound S-1 complexes indicates that the shape of S-1 in S-1–ADP and S-1–ADP–P_i states significantly differs from the shape of S-1 in nucleotide-free states [41].

It seems, our interpretation is in fair agreement with the experimental data obtained on protein solutions [26] and on S-1 subfragment [42], and it seems to be consistent with the model recently proposed by Rayment et al. [3].

We have performed the first modulated experiments in proteins as we know from the literature. We obtained from these data, information for the fine internal domain structural rearrangement during the different states of muscle contraction. $C_{p,total}$ gave practically same result as the conventional DSC, except of ADP state which should be carefully reinvestigated, but in $C_{p,modulated}$, we could not observe any sharp transition. It showed a stepwise change: before and after the melting region, it was nearly constant, and between 45 and 70°C, it decreased monotonically (Fig. 2). This supports the proposal that the C_p of biological macromolecules (proteins) should be temperature-dependent [15,16] in contrast to the recent opinions. To clarify this problem, we should perform experiments in the function of modulation frequency and amplitude too.

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