Journal of Thermal Analysis and Calorimetry, Vol. 64 (2001) 651–658

NUCLEOTIDE ANALOGUE INDUCES GLOBAL AND LOCAL CHANGES IN MUSCLE FIBRES

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Abstract

The effect of AMP.PNP on the thermal stability and dynamics of myosin head were investigated by using DSC and different spin label technique for chemically skinned muscle fibres prepared from rabbit. The thermal unfolding of the fibres in rigor, strong as well as weak-binding state showed a complex process characterizing at least three discrete domain regions with different stability (T_m=54, 58.4 and 62.3°C). The unfolding at 54°C refers to the catalytic domain of myosin, whereas transition at T_m=58.4°C represents the rod-like region. In the presence of AMP.PNP only the parameters of the last transition changed significantly (T_m=70.4°C) showing an increased interaction between actin and myosin heads being attached to actin. Measurements on MSL-fibres (labelled at Cys-707 of myosin) in the presence of AMP.PNP showed that about half of the cross-bridges dissociated from actin. This fraction had a dynamic disorder, the other population had the same spectral feature as in rigor. In contrast, on TCSL-fibres AMP.PNP increased the orientational disorder of myosin heads, a random population of spin labels was superimposed on the ADP-like spectrum showing conformational and motional changes in the internal structure of myosin heads. ST EPR measurements reported increased rotational mobility of spin labels in the presence of AMP.PNP. The DSC and EPR results suggest that in the presence of AMP.PNP the attached heads have the same global orientation as in rigor, but the internal structure undergoes a local conformational change.

Keywords: DSC, EPR, MSL, rabbit muscle fibres, TCL, thermal stability

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]. We

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1418–2874/2001/ $ 5.00
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have extended the experiments to study the fibre system prepared from psoas muscle of rabbit in rigor, strongly binding (ADP) and weakly binding states (AMP.PNP) of myosin to actin. 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 [7]. The changes in the 50 kDa domain might effect the segment of the 20 kDa domain that contains the essential sulfhydryl groups.

Spectroscopic probes are widely used in biological systems to get information about orientation and rotational motion of proteins. In muscle fibre studies, the maleimide-based nitroxides (MSL) are usually attached to the reactive sulfhydryl sites (Cys 707, Cys 697) of myosin heads, and two different orientations are tested: the longer axis of the fibres is oriented parallel and perpendicular to the magnetic field [8]. The different labels have different chemical structures and a different attaching linkage, therefore, it is reasonable to use different labels to understand the molecular motion of the head region of myosin in the presence of nucleotides [9].

In this report, we studied the effect of MgADP and AMP.PNP on the dynamics and orientation of myosin head using an isothiocyanate spin label and EPR spectroscopy. In order to find correlation between local and global structural changes in the intermediate states of the ATPase cycle, the spectroscopic technique was combined with conventional DSC measurements that report domain stability and interactions.

Experimental

Materials and methods

Preparation of muscle fibres

Glycerol-extracted muscle fibre bundles were prepared from psoas muscle of rabbit. Small stripes of muscle fibres (20–25 mm in length and 1 mm in diameter) were stored after osmotic shocks in 50% V/V glycerol and rigor solution (100 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 10 mM histidine·HCl, pH 7.0) in refrigerator at –18°C for 3 days to 1 month before use.

DSC measurements

Thermal unfolding was monitored by a Setaram Micro DSC-II calorimeter. All experiments were carried out between 10 and 80°C with a 0.3°C min^{-1} scan rate. Conventional Hastelloy batch vessels were used during the thermal transition experiments with 850 μl sample volume in average. Rigor buffer was used as a reference sample. The sample and reference vessels were equilibrated with a precision of ±0.1 mg. There was no need to perform any correction from the point of view of heat capacity between the sample and reference vessels.

Evaluation of DSC scans

The repeated scan of denaturated sample was used as baseline reference which was subtracted from the original DSC scan. Calorimetric enthalpy was calculated from the area under the heat absorption curves using two points setting Setaram peak integration.

Spin-labelling of muscle fibres

Spin-labelling of muscle fibres was performed in relaxing medium (rigor solution plus 2 mM pyrophosphate at pH 7.0) with about one mole of 4-maleimido-TEMPO (MSL) or 4-isothiocyanato-TEMPO (TCSL) to one mole myosin for 10 or 20 min at 0°C. In labelling buffer KCl was replaced by potassium-propionate. After spin-labelling the fibre bundles were washed in rigor buffer to remove the unreacted labels.

EPR measurements

The EPR measurements were taken with an ESP 300 E (Bruker, Germany) X-band spectrometer. For conventional EPR technique 100 kHz field modulation (0.2 mT amplitude) and 20 mW microwave power were used. Second harmonic absorption, 90° out-of-phase spectra were recorded with 50 kHz field modulation and detection at 100 kHz out-of-phase. The microwave power was 63 mW which corresponds to an average microwave field amplitude of 0.025 mT in the central region of the flat cell.

Results and discussion

DSC measurements

The unfolding of the supramolecular structure in muscle fibres by heat is a complex process. Earlier data on muscle proteins suggest at least three or four transitions in the temperature range examined [13–15]. The experimental patterns on muscle fibres in different states exhibited three large transitions, whereas the detailed analysis by computer resulted in four transitions. The endotherms in all states are believed to correlate mainly with T_ms of the principal domains of the main proteins, myosin and actin. In the absence of nucleotides (rigor): T_{m1}=54.2°C, T_{m2}=59.2 and T_{m3}=67.8°C (Fig. 1). Deconvolution procedure resulted in a fourth transition at 63.2°C.

Addition of nucleotides (ADP, AMP.PNP) produces conformational changes in the multisubunit structure of myosin and affects the actin-myosin interaction as revealed by EPR and other spectroscopic studies [16, 17]. The transition temperatures of the thermal unfolding depend on the nucleotides (Table 1). MgADP affected moderately the transition temperatures, but the conversion from a strongly attached state of myosin to actin to a weakly binding state is accompanied with an increase of the highest transition temperature from 67.8 to 70.4°C (Fig. 2).

The results of melting of myosin and actin in the supramolecular structure of muscle fibres cannot be interpreted easily. According to the measurements the first two transitions were little affected by the binding of nucleotides. Earlier measure-

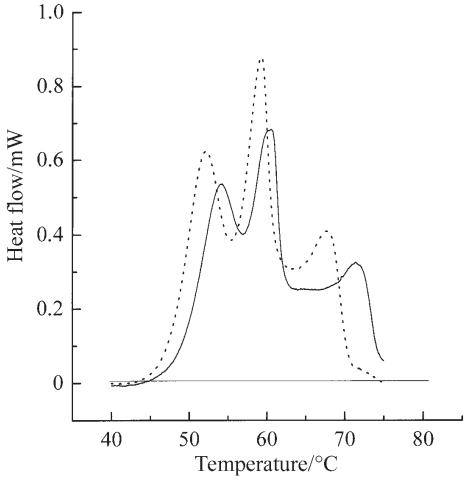

Fig. 1 DSC curves of muscle fibres in rigor (dotted line) and MgADP state (solid line). The fibres were incubated in rigor buffer plus 5 mM MgADP. 50 μM diadenosine pentaphosphate was added to the buffer solution to inhibit the myokinase activity

ments reported that the most labile part of myosin was the 50 kDa segment which binds actin, therefore it is believed that the first thermal transition can be assigned to the 50 kDa domain even in muscle fibres [18]. This transition might involve the unfolding of the subfragment-2 domain and the rod part of the myosin as well [19].

Table 1 DSC results on muscle fibres. Muscle fibres were measured in different state of the ATP hydrolysis cycle in the temperatures in range of 40–75°C. The fibres were bathed in the appropriate buffer solution containing the nucleotide (5 mM ADP or AMP.PNP) at 0°C for 15 min before measurement. DSC curves were analyzed using the PeakFit 4.0 program from SPSS Corporation

	Experimental results			Results of decomposition		
	T_1/°C	T_2/°C	T_3/°C	T_1/°C	T_2/°C	T_3/°C
Rigor	52.2	59.3	67.8	52.39	58.83	67.94
ADP	53.0	57.7	67.5	53.19	57.61	67.08
AMP.PNP	52.7	57.7	70.4	52.82	57.47	69.87

In the presence of AMP.PNP the largest change was measured at the highest temperature transition. It suggests that the dissociation induced by AMP.PNP reduces the actin-myosin interaction, which leads to a significant decrease of the contribution of the enthalpy at the 65.2°C transition (Fig. 2). On the other hand, the increased thermal stability of the dissociated globular heads with the bound nucleotide appears in the last transition.

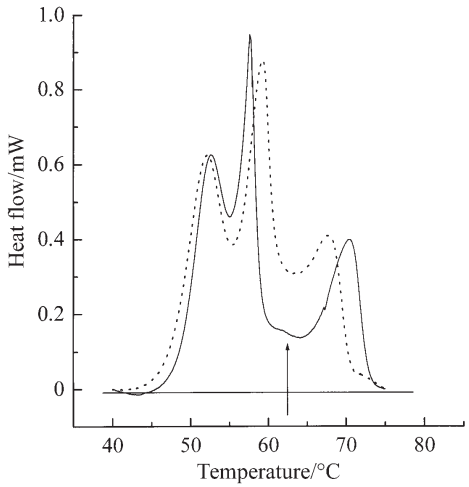

Fig. 2 DSC curves of muscle fibres in rigor state (dotted line) and MgAMP.PNP state (solid line). The fibres were incubated in rigor buffer containing 5 mM MgAMP.PNP for 15 min at 0°C before measurement

EPR measurements

The EPR spectra of spin-labelled fibres showed a strong dependence of orientation in the absence of nucleotide (rigor state, strong binding state of myosin heads to actin), implying that almost all myosin heads were attached to actin [11]. Both labels, MSL

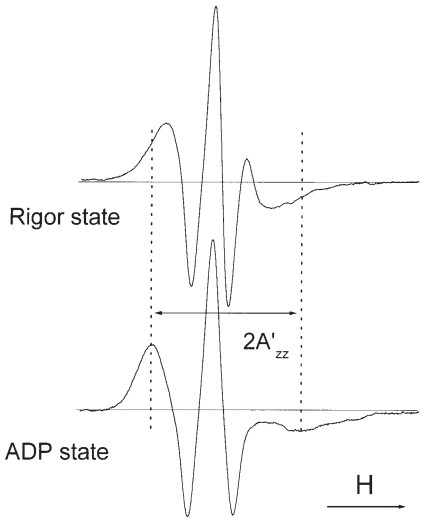

Fig. 3 EPR spectra of TCSL-fibres in rigor and MgADP state. The long axis of the fibres was oriented parallel to the laboratory magnetic field. Addition of ADP induced large change of the hyperfine splitting ($2A'_{zz}$). The field scan was 10 mT

and TCSL reflected the orientation dependence of myosin heads, but the parameters of the distribution density function for label orientations were different [9]. The mean angle (ϑ) of the label orientation and the angular spread (σ) of the orientation differ each other; ϑ(MSL)=82°, ϑ(TCSL)=75°, σ(MSL)=10° and σ(TCSL)=16°. The two labels have different attaching linkage, ST EPR measurements revealed that MSL were more rigidly attached to the protein, than TCSL. In contrast to MSL-fibres, the addition of MgADP to the buffer solution (5 mM ADP plus 50 μM diadenosine pentaphosphate to inhibit myokinase) induced local conformational changes, which resulted in significant alteration of the orientation dependence evidencing that TCSL label was sensitive to the change of the local conformation (Fig. 3). The mean angle of the orientation distribution changed from 75 to 56° and the angular spread increased (σ=28°). The larger hyperfine splitting constant $2A'_{zz}$ in ADP state clearly indicated the change of the label orientation. The addition of MgATP and orthovanadate in 5 mM final concentration to the buffer solution weakens the binding of myosin to actin or detache the myosin heads (weakly binding state of myosin to actin) from actin [12]. This structural rearrangement is accompanied by increased rotational motion of the myosin heads. In this case almost no orientation dependence can be detected (Fig. 4), the spectrum resembles the spectrum of randomly oriented spin labels.

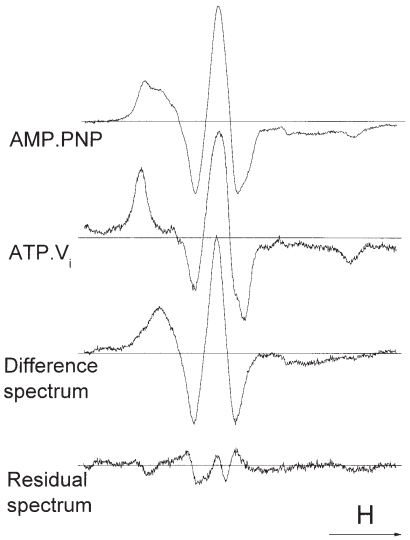

Fig. 4 EPR spectra of TCSL-fibres in AMP.PNP state. The long axis of the fibres was oriented parallel to the laboratory magnetic field. The EPR spectrum of TCSL-fibres in the presence of MgAMP.PNP was superposition of two spectra. From this spectrum the EPR spectrum of fibres in ATP.V_i state was subtracted. The difference spectrum was almost identical with the spectrum obtained in ADP state. At bottom the difference of the ADP-like spectrum and the ADP spectrum (Fig. 3) in ADP is given

In the presence of non-hydrolysable analogue AMP.PNP, the conventional EPR spectra showed large changes in the ordering of the probe molecules in fibres [10]. The orientation dependence of the ordered population depended on the chemical structure of the spin labels. Spectrum deconvolution resulted in two populations; about 50% of labels belonged to the ordered fraction, and 50% of labels was randomly oriented. From earlier observations it was concluded that the ordered fraction could not be distinguished from the rigor population [10]. Our results on AMP.PNP-fibres using TCSL suggest that about half of the heads represents a disordered population with reduced rate of rotational motion, but the myosin heads which exhibited high degree of order were in the strongly binding ADP state, the heads being attached to actin differ from that of rigor (Fig. 4). Subtracting the ADP.V_i spectrum from the AMP.PNP spectrum, the difference spectrum approximates quite well the ADP spectrum. Residual spectrum in Fig. 4 shows the difference of the two ADP spectra. The binding of AMP.PNP might induce change in the orientation of the protein segment that holds that holds the label by rotation resulting in another conformationally stable state. ST EPR spectra of MSL-fibres in AMP.PNP state reported increased rotational motion (Fig. 5). The ratio of the low-field diagnostic peak (L''/L) markedly decreased, implying the detachment of myosin heads from actin. The effective rotational correlation time could be estimated to be about 100 μs.

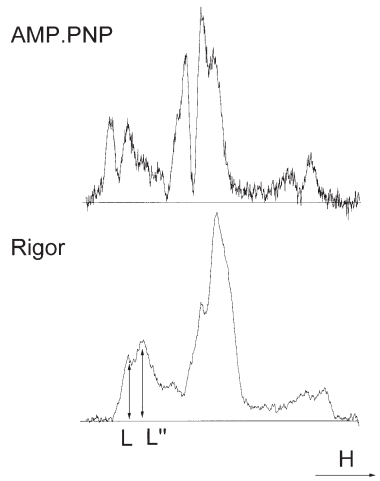

Fig. 5 ST EPR spectra of MSL-fibres in rigor and AMP.PNP state. The ratio of the two diagnostic peaks (L'' and L) decreases in AMP.PNP state, evidencing the reduced rate of the rotational motion

To sum up, the data support that nucleotides and nucleotide analogues induce changes in the dynamical state of myosin heads [20]; according to the EPR results the two strongly binding states, the ADP-state and rigor state differ from each other in local conformation. The differences in the melting temperatures measured by DSC

clearly indicate that nucleotide binding can produce significant alterations in the global structure of myosin head [21, 22].

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This work was supported by grants from the National Research Foundation Hungary (OTKA CO-123 and T 030248 for J. B. as well as CO-272 for D. L.) and from the Inco Copernicus (EU Erbic 15CT960821 for D. L.).

References

1 R. W. Lymn and E. W. Taylor, Biochemistry, 10 (1971) 4617.
2 H. E. Huxley, Cross-Bridge Mechanism in Muscle Contraction (H. Sugi and G. H. Pollack Eds) University of Tokyo Press, Tokyo 1979, p. 391.
3 I. Rayment, H. M. Holden, M. Whittaker, C. B. John, M. Lorentz; K. C. Holmes and R. A. Milligan, Science, 261 (1993) 58.
4 A. Orlova and E. H. Egelman, J. Mol. Biol., 232 (1993) 334.
5 A. Orlova and E. H. Egelman, J. Mol. Biol., 245 (1995) 582.
6 B. Brenner and E. Eisenberg, Cardiac Energetics Basic Mechanism and Clinical Applications (in Supplement to Basic Research in Cardiology, Eds R. Jacob, Hj. Just and Ch. Holubarsch), Springer, New York 1987, 82.2, p. 3.
7 A. J. Fischer, C. A. Smith, J. Thoden, R. Smith, K. Sutoh, H. M. Holden and J. Rayment, Biophys. J., 68 (1995) 19S.
8 P. G. Fajer, E. A. Fajer, J. J. Matta and D. D. Thomas, Biochemistry, 29 (1990) 5865.
9 J. Belágyi, I. Frey and L. Pótó, Eur. J. Biochem., 224 (1994) 215.
10 P. G. Fajer, E. A. Fajer, N. J. Brunsvold and D. D. Thomas, Biophys. J., 53 (1988) 513.
11 D. D. Thomas and R. Cooke, Biophys. J., 32 (1980) 891.
12 C. C. Goodno, Proc. Natl. Acad. Sci. USA, 76 (1979) 2620.
13 D. Lőrinczy and J. Belágyi, Thermochim. Acta, 196 (1997) 161.
14 D. Lőrinczy, B. Gaszner and J. Belágyi, High Temp. High Press., 30 (1998) 119.
15 D. Lőrinczy and J. Belágyi, Thermochim. Acta, 343 (2000) 27.
16 S. B. Marston, C. D. Rodger and R. T. Tregear, J. Mol. Biol., 104 (1976) 263.
17 K. Ajtai, A. Ringler and T. P. Burghardt, Biochemistry, 31 (1992) 207.
18 A. Setton and A. Muhlrad, Arch. Biochem. Biophys., 235 (1985) 411.
19 L. King and S. Lehrer, Biochemistry, 28 (1989) 3489.
20 K. Franks-Skiba and R. Cooke, Biophys. J., 68 (1995) 142S.
21 D. I. Levitsky, V. L. Shnyrov, N. V. Khvorov, A. E. Bukatina, N. S. Vedenkina, E. A. Permyakov, O. P. Nikolaeva and B. F. Poglazov, Eur. J. Biochem., 209 (1992) 829.
22 A. A. Bobkov and D. I. Levitsky, Biochemistry, 34 (1995) 9708.