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Microcalorimetric study on the interaction of F-actin with myosin and its proteolytic fragments ☆

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Abstract

The heat effect produced in the interaction of rabbit muscle F-actin with myosin and its proteolytic fragments (HMM, S_1) was measured with an MS-80 Calvet microcalorimeter (Setaram, France). An improved reaction cell of the microcalorimeter was used. There were three thermokinetic peaks in the measured curve of the interaction of myosin with F-actin in the absence of ATP, which were exothermic, endothermic and exothermic in order of appearance. When ATP was added to F-actin before mixing with myosin, the curve remained similar in feature to that in the absence of ATP. But the first peak (exothermic) became smaller and the second and third peaks became larger. There was only one exothermic peak in the measured curve of the interaction between S_1 and F-actin. But ATP caused big changes in the curve of their interaction. With addition of ATP to F-actin solution before it was mixed with S_1, there were four thermokinetic peaks in the curve which were endothermic, exothermic, endothermic, and exothermic in order of appearance. The measured curves of interaction between HMM and F-actin in the presence and absence of ATP were quite similar to that of interaction between S_1 and F-actin.

Keywords: ATP; F-actin; Microcalorimetry; Myosin

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1. Introduction

Interaction between F-actin and myosin coupled with hydrolysis of adenosine triphosphate (ATP) is the fundamental process for generation of the force to drive muscle contraction and other actin-dependent intracellular cell motility, which is of much physiological significance. The kinetics of interaction between F-actin and myosin have been characterized in great detail [1–5]. However, its thermodynamics are not clear. The interaction between F-actin and myosin is performed through a weak bonding force. Therefore, its heat effect is very small and it is quite difficult to measure the heat produced in the process of their interaction. We have improved that reaction cell of the Calvet MS-80 Microcalorimeter (Setaram, France) in order to measure this small heat effect.

In this study, we investigated thermograms of interaction of muscle F-actin with myosin, heavy meromyosin (HMM) and myosin sub-fragment 1 (S_1), in the presence and absence of ATP, by means of calorimetry.

2. Experimental

2.1. Preparation of proteins

Actin was purified by the method of Spudich and Watt [6]. Rabbit myosin was purified according to the method of Kielly and Harrington [7]. F-actin was dissolved in a buffer containing 20 mM Tris, 0.1 mM $CaCl_2$, 0.75 mM DTT, 0.05% NaN_3 and 0.1 mM ATP with a pH value of 8.0. Actin was polymerized by addition of KCl and $MgCl_2$ to concentrations of 100 mM and 2 mM respectively. ATP-free F-actin was prepared through dialysis of the above-polymerized actin against the same buffer without ATP in which 100 mM KCl and 2 mM $MgCl_2$ were present. Heavy meromyosin (HMM) was prepared by digestion of myosin with trypsin using the method of Margossian and Lowey [8]. Myosin sub-fragment 1 (S_1) was prepared by digestion of myosin with papain according to Margossian and Lowey [8]. HMM and S_1 were further purified through DEAE cellulose chromato-graphy as described by Margossian and Lowey [8].

2.2. Determination of concentrations of proteins

Concentrations of protein were determined spectrophotometrically, using the following extinction coefficients: $E_{290}^{1\%} = 6.2\ cm^{-1}$ for actin, $E_{280}^{1\%} = 5.3\ cm^{-1}$ for myosin, $E_{280}^{1\%} = 8.1\ cm^{-1}$ for S_1 and $E_{280}^{1\%} = 6.0\ cm^{-1}$ for HMM.

2.3. Assay of ATPase activity

ATPase activities of myosin, HMM and S_1 were determined by the method of Margossian and Lowey [2]. P_i hydrolyzed from ATP was determined by the method of Le Bal et al. [9].

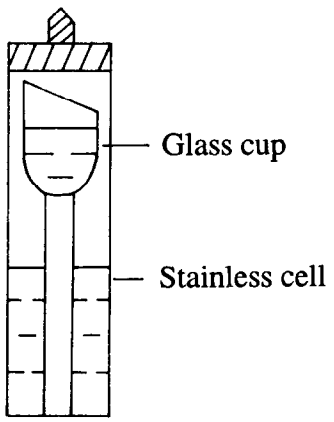

Fig. 1. A sketch of the improved reaction cell.

2.4. Measurement of heat

The improved reaction cell of the Calvet MS-80 microcalorimeter is shown in Fig. 1. Myosin, HMM or S_1 (3 ml) was added to the stainless steel cell and 0.5 ml of F-actin was added to the glass cup in this experiment. In the control cell, F-actin was replaced by buffer of F-actin. The reaction was started by turning the microcalorimeter down and up five times ($5 \times 180°$) when the machine came to thermal equilibrium. The experimental temperature was 25°C.

3. Results

3.1. Interaction of myosin with F-actin

Myosin binds to F-actin in the absence of ATP to form actomyosin. There were three thermokinetic processes in the formation of actomyosin, exothermic, endothermic and exothermic in order of appearance (Fig. 2A). The first peak in the measured curve is sharp. It takes about 40 min to complete the thermokinetic processes which implies that the thermodynamics of binding of myosin to F-actin is very complicated. We believe that conformational changes in myosin induced by F-actin, and thermal bending of both myosin and F-actin are involved. The first thermokinetic process (exothermic peak) corresponds to the combination of myosin with F-actin. It is very fast and corresponds with the conclusion of other techniques such as viscometry. Fig. 2A shows that this thermodynamic process lasts about 3 min.

When ATP was added to F-actin to a concentration of 1 mM before it was mixed with myosin, with other conditions remaining the same, the measured cuve showing the binding of myosin to F-actin was similar in features to that recorded in the absence of ATP (Fig. 2B). But the first exothermic peak became smaller and the

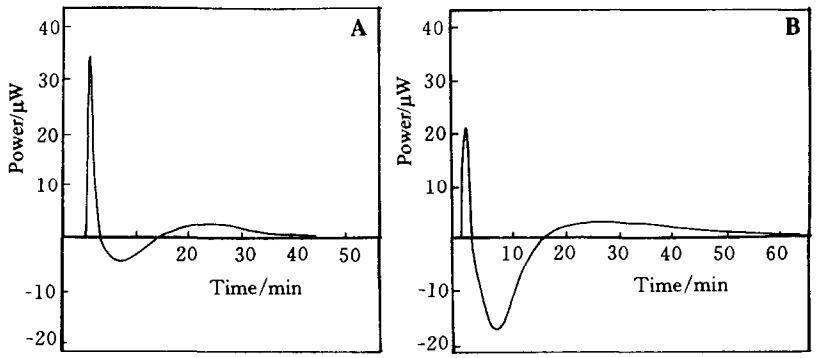

Fig. 2. Measured curves of interaction between F-actin and myosin in the absence (A) and presence (B) of 1 mM ATP at 25°C. Myosin (3.37 mg ml^{-1}) was dissolved in buffer containing 20 mM Tris and 500 mM KCl with a pH of 8.0. F-actin (0.16 mg ml^{-1}) was dissolved in buffer containing 20 mM Tris, 0.1 mM $CaCl_2$, 0.05% NaN_3, 0.75 mM DTT, 2 mM $MgCl_2$ and 100 mM KCl with a pH of 8.0. The amplification of the microcalorimeter was 10 μV.

second and third peaks became larger. It is known that ATP reduces the affinity between F-actin and myosin. So the linking force between them becomes weakened. The fact that the first thermokinetic peak became smaller is in agreement with this. Fig. 2B shows that ATP enhanced the second and third thermokinetic processes. We assayed ATPase activity of myosin in both control and reaction cells. With our experimental conditions, the activity of myosin in the reaction cell was a little higher than that in the control cell (data not shown). Besides, the measured curve of hydrolysis of ATP by myosin is a simple exothermic process (Fig. 3). We think that the change in the curve is not caused by hydrolysis of ATP. We propose that ATP enhances the conformational change of the mysoin molecule when it interacts with F-actin.

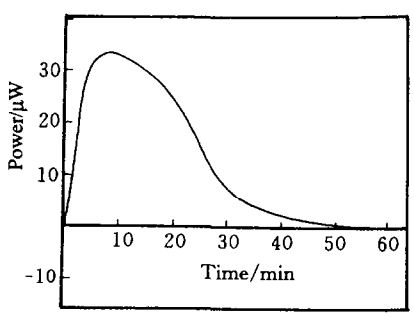

Fig. 3. Measured curve of hydrolysis of ATP by myosin at 25°C. Concentration of ATP was 1 mM. Myosin (3.37 mg ml^{-1}) was dissolved in buffer containing 20 mM Tris and 500 mM KCl with a pH of 8.0. The amplification of the microcalorimeter was 25 μV.

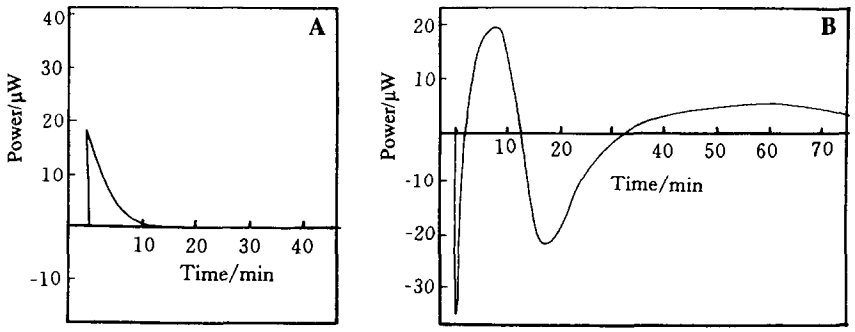

Fig. 4. Measured curves of interaction between F-actin and HMM in the absence (A) and presence (B) of 1 mM ATP at 25°C. HMM (2.43 mg ml^{-1}) was dissolved in buffer containing 20 mM Tris and 100 mM KCl with a pH of 8.0. F-actin (0.60 mg ml^{-1}) was dissolved in buffer containing 20 mM Tris, 0.1 mM $CaCl_2$, 0.05% NaN_3, 0.75 mM DTT, 2 mM $MgCl_2$ and 100 mM KCl with a pH of 8.0. The amplification of the microcalorimeter was 25 μV.

3.2. Interaction of HMM with F-actin

HMM has been used by many authors to study the mechanism of interaction between actin and myosin because of its ready solubility in solutions of low ionic strength. It has a strong affinity for F-actin in the absence of ATP, forming acto.HMM complex. The interaction of HMM with F-actin is a simple exothermic process (Fig. 4A). When ATP was added to F-actin solution before it was mixed with HMM, a big change took place in the measured curve of interaction between HMM and F-actin (Fig. 4B). There are four thermokinetic peaks in the curve, endothermic, exothermic, endothermic and exothermic in order of appearance. This implies that the thermodynamics of the process are very complicated. We believe that this curve will be very useful for elucidation of the interaction between F-actin and HMM because ATP causes big changes in the measured curve. The curve of hydrolysis of ATP by HMM (not shown) was similar to that in Fig. 3. The activity of HMM ATPase in the reaction cell was a little higher than that in the control cell. Therefore, the effect of hydrolysis of ATP on the measured curve is negligible.

3.3. Interaction of S_1 with F-actin

S_1 is a very useful sub-fragment of myosin for studying the interaction of actin with myosin. The kinetic analysis of binding of S_1 to F-actin was carried out in detail. Our study showed that binding of S_1 to F-actin in the absence of ATP was an exothermic process (Fig. 5A). When ATP is present, a big change occurs in the measured curve (Fig. 5B), in which there are four thermokinetic peaks, endothermic, exothermic, endothermic and exothermic in order of appearance. The activity of S_1 ATPase in the control and reaction cells was also assayed. The S_1 ATPase activity in the reaction cell was also a little higher than that in the control cell. The curve of hydrolysis of ATP by S_1 (not shown) was similar to that in Fig. 3. Thus

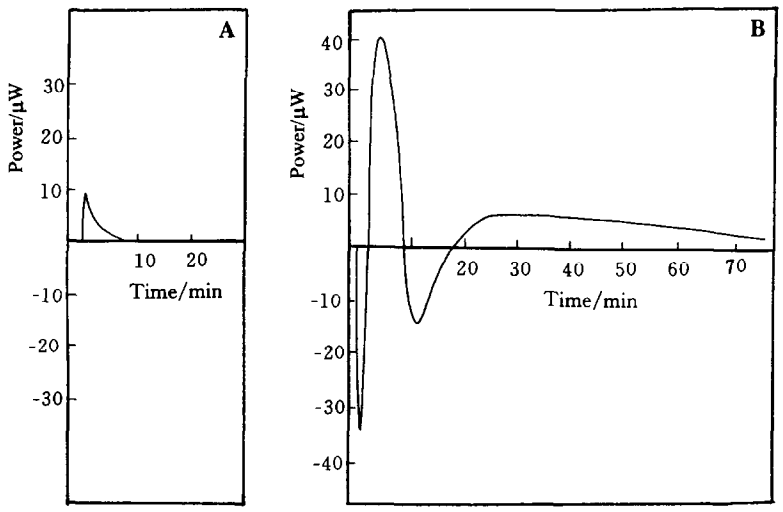

Fig. 5. Measured curves of interaction between F-actin and S_1 in the absence (A) and presence (B) of 1 mM ATP at 25°C. S_1 (1.12 mg ml^{-1}) was dissolved in buffer containing 20 mM Tris and 100 mM KCl with a pH of 8.0. F-actin (0.60 mg ml^{-1}) was dissolved in buffer containing 20 mM Tris, 0.1 mM $CaCl_2$, 0.05% NaN_3, 0.75 mM DTT, 2 mM $MgCl_2$ and 100 mM KCl with a pH of 8.0. The amplification of the microcalorimeter was 25 μV.

any influence of hydrolysis of ATP by S_1 was ruled out. It can be seen from Fig. 5 that ATP induces a conformational change in S_1 when it interacts with F-actin. In addition to this, the heat effect of interaction between S_1 and F-actin increased markedly with addition of ATP.

4. Discussion

Our results suggest that the thermodynamics of interaction of F-actin with myosin and its sub-fragments is very complicated, consisting of several thermokinetic processes in the presence of ATP. The measured curves of the interaction between F-actin and HMM are quite similar to those of the interaction between F-actin and S_1 in the presence and absence of ATP. But they are different from the curve of interaction between F-actin and myosin. As we know, HMM with two heads and S_1 with only one head are both sub-fragments of myosin. HMM is smaller than myosin but larger than S_1 in molecular structure. However, their thermokinetic properties are very similar when they interact with F-actin. However, myosin, which is made up of light meromyosin (a tail rod) and HMM, shows that its thermokinetic property of interacting with F-actin is appreciably different from that of HMM and S_1. We believe that the structural basis responsible for the difference in these thermokinetic properties is the tail rod of myosin, namely, light meromyosin. To date, the role of the rod tail of myosin is not very clear. Tsong et al. [10] reported that S_2 can change reversibly from helix to coil and back fast

enough to be compatible with the mechanism of muscle contraction. Such a transition, however, appears to correspond to passive elasticity, possibly accounting for the elastic element rather than for the active element of a force-generating mechanism. Our results show that LMM or S_2 greatly influence the measured curves of interaction between F-actin and myosin. We propose tentatively that LMM or S_2 plays an active role in the force-generating mechanism.

As we described here, ATP causes large changes in the measured curves of interaction of F-actin with myosin, HMM, and S_1. We think that this is partially due to the conformational change in the head of myosin. A variety of studies have shown that the binding of F-actin causes a local conformational change in the head of S_1 or HMM [11–13]. These studies also show that conformational change increases if nucleotides are present. In addition to this, the flexibility of F-actin may possibly be related to changes in the measured curves caused by ATP. Although the flexibility of F-actin is not generally accepted, studies over the years do prove that F-actin is flexible when it binds with globular protein molecules. F-actin can undergo a bending movement for an apparent period for ten minutes. Furthermore, the bending movement is activated by hydrolysis of ATP [14]. Our results may prove that F-actin is flexible.

Our research shows that microcalorimetry is a new and sensitive method for investigating the interaction between actin and myosin. It has provided us with information on energetic transduction which may be required in understanding the mechanism of force generation for myosin. We think that this study can be extended as follows. (1) Calculating the values of the thermodynamic parameters (ΔG, ΔH, ΔS) of formation of actomyosin, acto.S_1 and acto.HMM. The changes in enthalpies will indicate whether each head of HMM has the same linking force on binding the F-actin or whether the ΔH of HMM binding to F-actin is twice as much as that of S_1 binding of F-actin. Margossian and Lowey [2] tried to obtain the data by van't Hoff plots of equilibrium constants (K_a) at different temperatures. But they found that the error in the ΔH of formation of acto.HMM as determined by a van't Hoff plot was too large to draw any definite conclusions. (2) Determining the effect of modification of myosin, HMM or S_1 on the measured curves of their interaction with F-actin. (3) Determining the effect of ionic strength and temperature on the measured curves and the enthalpy changes of the interaction of F-actin with myosin and its proteolytic fragments. (4) Understanding the mechanism of each thermodynamic process in the curves. Together, all these will add to our knowledge of the interaction between actin and myosin and will help to elucidate the energetics of force generation by myosin.

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