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Parking problem and negative cooperativity of binding of myosin subfragment 1 to F-actin

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ABSTRACT

Previously we provided evidence that myosin subfragment 1 (S1) can bind either one (state 1) or two actin monomers (state 2) in solution and in muscle fiber. Here we present results of the kinetics study of binding of S1 to F-actin labeled with fluorescent dye pyrene. A transition from state 1 to state 2 depends on probability that the second actin is free, which is high when molar ratio of S1/actin (R) is less than 0.5, and it decreases dramatically when $R > 2.0$ due to the parking problem. The kinetics data obtained at different molar ratios were well fitted by two binding states model. The sequential binding of myosin head initially with one actin monomer and then with the second actin monomer in F-actin can play a key role in force generation by actin–myosin and their directed movement.

1. Introduction

A muscle contraction results from the sliding of actin and myosin filaments induced by ATP-driven cyclic interaction of myosin with actin [1,2]. Lymn and Taylor showed that the binding of ATP to myosin induces its dissociation from F-actin followed by ATP hydrolysis in the detached state and, then, re-binding of myosin to F-actin and release of hydrolysis products [3]. Andrew Huxley suggested that binding of myosin to F-actin occurs in several steps by transition from weak to strong contacts between myosin and actin (rolling of myosin on actin filament) [4]. We showed previously that myosin head, called subfragment 1 (S1), can bind either one or two actin monomers [5–8]. The ability of S1 to bind two actins was independently confirmed by other laboratories as well [9–12]. When S1 molecules are in a molar excess of actin molecules then each bound S1 interacts predominately with one actin (1:1 complex). When actin is in excess, S1 molecules bind predominately two actin monomers (1:2 complex) [5–7]. Using the fast quench crosslinking technique we demonstrated that the initial contact occurred between loop of 627–637 residues of S1 with N-terminus of actin monomer and later loop of 567–575 residues of S1 binds to N-terminus of the second actin monomer [13]. Here we present the results of kinetics study of S1 binding with F-actin, which confirm that binding occurs in two steps and transition from state 1 to state 2 is very fast and its rate is comparable with the myosin power stroke time (~10 ms) in actively contracting muscle.

2. Materials and methods

2.1. Materials

Myosin subfragment 1 was prepared by digestion of myosin (Cytoskeleton, Inc.) by chymotrypsin and separation of two S1 isoforms (S1A1 and S1A2, A1 and A2 are alkali light chains 1 and 2, respectively) on DEAE column as described previously [14]. Actin (Cytoskeleton, Inc.) was labeled with N-(1-pyrenyl) iodoacetamide (Invitrogen, Inc.) as described before [7]. The degree of labeling actin with pyrene was 95–100%. Actin–pyrene was passed through sephadex G-50 spin column equilibrated with 0.2 mM $CaCl_2$, 2 mM Trsi-HCl pH 7.5 buffer solution and then polymerized by adding 50 mM KCl, 2 mM $MgCl_2$ and equal molar amount of phalloidin (Invitrogen, Inc.).

2.2. Kinetic measurements

Fluorescence kinetics measurements were carried out on a SFM-300 mixing apparatus connected to a MOS-450 spectrometer (Biologic, Inc.) under temperature control. One syringe was loaded with F-actin–pyrene and the other with S1. All solutions were degassed several minutes under a vacuum before loading into the syringes to minimize air bubbles. Experiments were done at different molar ratios of S1/actin. The binding of S1 to F-actin was monitored by measuring fluorescence signal from F-actin–pyrene at 410 nm with excitation at 368 nm. Each kinetic curve was recorded several times (~10) and then averaged, excluding the first 2–3 shots. The measured signal was normalized by the fluorescence signal monitored at mixing of F-actin–pyrene with buffer solution

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at the same conditions. All experiments were done in 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 2 mM Tris–HCl buffer pH 7.5 at 20 °C.

2.3. The two-statebinding model

The two-state model of actin-myosin interaction was proposed by us earlier [7]. We assumed that binding of myosin subfragment S1 (M) to F-actin (A) occurs in two steps: (i) initially S1 binds one actin to form complex (AM) – state 1:

$$A + M \underset{k_{-1}}{\overset{k_{+1}}{\leftrightarrow}} AM$$

ii) then it binds the second actin located in the same strand if it is free to form complex (AMA) – state 2 (Fig. 1)

$$AM + M \underset{k_{-2}}{\overset{k_{+2}}{\leftrightarrow}} AMA$$

where k_{+1}, k_{+2}, k_{-1} and k_{-2} are the kinetic constants and the equilibrium adsorption constant for state 1 is

$$K_1 = \frac{k_{+1}}{k_{-1}} \tag{1}$$

the equilibrium constant of transition from state 1 to state 2 is:

$$K_2 = \frac{k_{+2}}{k_{-2}} \tag{2}$$

The rate of adsorption of S1 for state 1 is:

$$v_1 = k_{+1} M_F A_F - k_{-1} M_1 \tag{3}$$

where A_F, M_F and M_1 are the concentration of unoccupied actin, the concentration of free S1 in the solution and the amount of bound S1 in state 1, respectively.

$$A_F = A - M_1 - 2M_2 \tag{4}$$

$$M_F = M - M_1 - M_2 \tag{5}$$

where A, M and M_2 are the total amount of actin, myosin and the amount of bound S1 in state 2, respectively.

The rate of transition from state 1 to state 2 is given by:

$$v_2 = k_{+2} M_1 P_F - k_{-2} M_2 \tag{6}$$

where P_F is the probability of the existence of free site next to a given singly bound S1. Any free actin or any acto-S1 complex can be the nearest neighbor to a single-actin bound S1. The number of ways for free actin to be in this place is equal to a number of free actins, A_F. The number of ways of all occupations (by free actin or by acto-S1) of this place is equal to the sum of free actins and acto-S1 complexes, i.e., $A_F + M_1 + M_2$. Thus the probability P_F is:

$$P_F = \frac{A_F}{A_F + M_1 + M_2} = \frac{A - M_1 - 2M_2}{A - M_2} \tag{7}$$

The change of the number of myosin molecules in different states with time is determined by

$$\frac{dM_1}{dt} = v_1 - v_2 \tag{8}$$

$$\frac{dM_2}{dt} = v_2 \tag{9}$$

The relative concentrations of adsorbed S1 on actin filaments are

$$m_1 = \frac{M_1}{A} \tag{10}$$

$$m_2 = \frac{M_2}{A} \tag{11}$$

and normalized concentration of free S1 in solution is given by:

$$m_F = K_1 M_F \tag{12}$$

Then, the set of adsorption equations that determine the changes of the number of myosin molecules in state 1 and 2 are

$$\frac{dm_1}{dt} = K_{-1} m_F (1 - m_1 - 2m_2) - k_{-1} m_1 - \frac{k_{+2} m_1 (1 - m_1 - 2m_2)}{1 - m_2} + k_{-2} m_2 \tag{13}$$

$$\frac{dm_2}{dt} = \frac{k_{+2} m_1 (1 - m_1 - 2m_2)}{1 - m_2} - k_{-2} m_2 \tag{14}$$

In experiment we record changes of fluorescence (F) of pyrene conjugated to actin when S1 binds to the actin. The fluorescence signal measured in experiments is a sum of fluorescence of free actin (f_0), actin occupied by S1 in state 1 (f_1) and in state 2 (f_2):

$$F = f_0 A_F + f_1 A_1 + f_2 A_2 \tag{15}$$

$$A_F = A - A_1 - A_2 = A(1 - m_1 - 2m_2)$$

$$\frac{F}{A} = f_0 - (f_0 - f_1) m_1 - 2(f_0 - f_2) m_2 \tag{16}$$

$F_0 = A f_0$ is the initial fluorescence signal at $t = 0$. During fitting we allow small (5%) variations for deviations in actin and S1 concentrations upon repetitive loading of actin and S1 in syringes. We applied nonlinear least squares fitting procedures using Levenberg–Marquardt algorithm to find the best fit of experimental kinetics curve measured for different S1/actin ratios. The variable parameters were equilibrium constants (K_1 and K_2) and backward kinetic constants ($k_{-1}$and k_{-2}) (the forward kinetic constants then could be calculated using Eq. (1) and (2)), and the normalized fluorescence of actin alone, bound to S1 in state 1 and state 2 f_0, f_1 and f_2. We allowed just slight variation of normalized fluorescence of actin in three different states, since from the additional experiments we had knowledge about the approximate values of these parameters. The calculations were carried out in MatLab7.0.

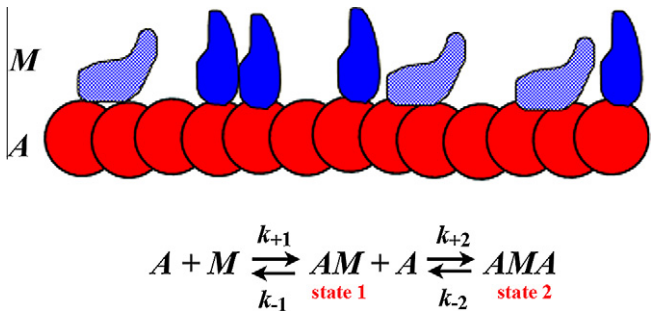

Fig. 1. Schematic diagram of the two-state binding of myosin subfragment 1 (M, shown in blue) to F-actin (A, shown in red) is presented. S1 can bind to one actin monomer in the vertical position to form AM complex, state 1 (dark blue) or to two actin monomers in the horizontal position to form AMA complex, state 2 (light blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results and discussion

We studied binding of S1 with F-actin–pyrene at different molar ratios (R = 0.5, 1, 2 and 4, where R = [S1]/[Actin]). The interaction of S1 with F-actin–pyr induces quenching of pyrene fluorescence [14,15]. The averaged kinetics plots for S1 binding to F-actin–pyr at various molar rations are shown on Fig. 2. All data were

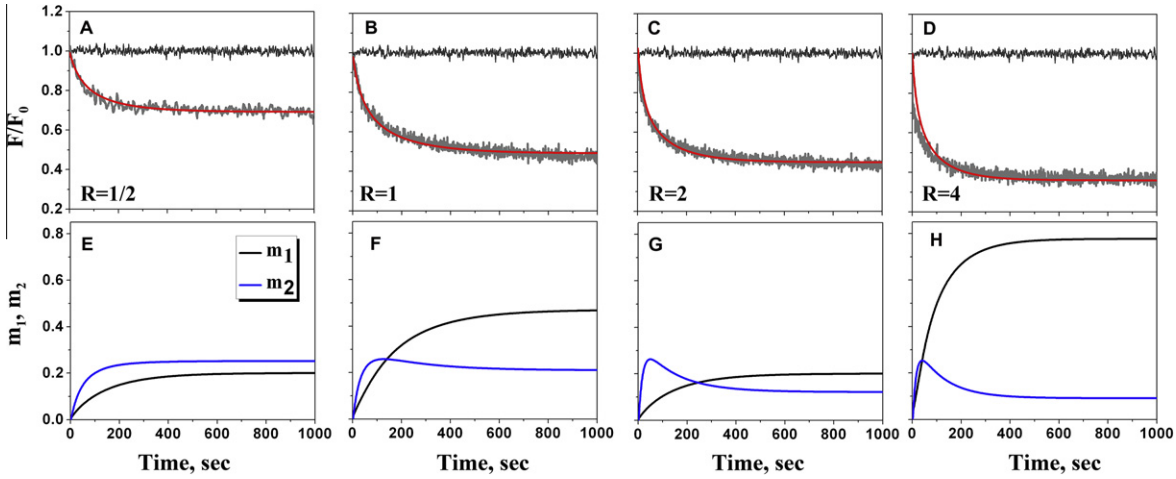

Fig. 2. Kinetics of binding of S1 to F-actin at different molar ratios. The time course of the quenching of fluorescence of F-actin–pyrene by binding of S1 is shown (A–D). The signal of F-actin–pyrene in absence of S1 is shown in dark grey) on (A–D). The data were fitted by using of the two-state binding model, theoretical curves are presented in red. Time dependences of fractional numbers of bound S1 in state 1 (m_1) and state 2 (m_2) predicted by the model for the experimental data obtained at different molar ratios are presented on (E–H). The equilibrium and kinetic constants obtained in the result of the fitting are given in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

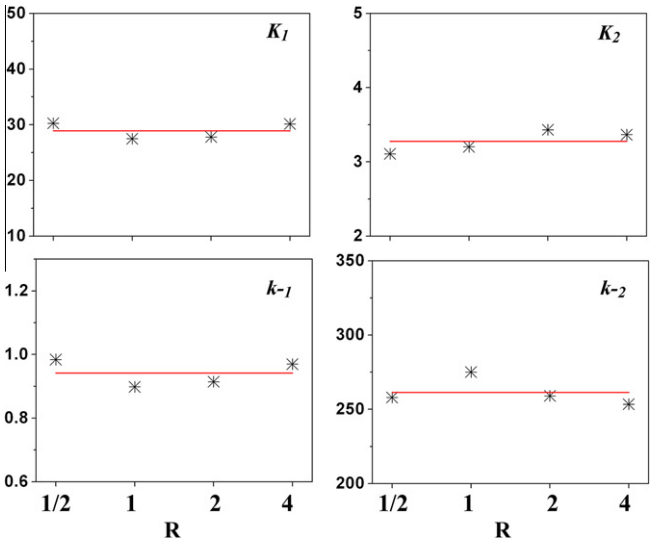

Fig. 3. The plots show values of the fitting parameters, such as equilibrium (K_1 and K_2) and kinetic (k_{-1} and k_{-2}) constants obtained in the result of fitting of each kinetic curve measured at various ratios of S1 to actin. The red line represents mean values, which are given in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

normalized for fluorescence signal measured when F-actin–pyr was mixed with buffer solution at the same conditions ($R = 0$). The data were fitted by the two-state model (theoretical curves shown in red on Fig. 2A–D). Due to the experimental error in samples concentrations after dilutions each parameter might slightly vary for each kinetic curve. Therefore, we performed fit of individual kinetic curves separately. The equilibrium and rate constants for binding of S1 and actin at different molar ratios were established (Fig. 3). There is just very slight difference between the same parameters obtained for various ratios of S1 and actin, the averaged values and standard deviations for each parameter are given in Table 1. Time dependences of fractional numbers of bound S1 in state 1 (m_1) and state 2 (m_2) predicted by the model for the experimental data obtained at different molar ratios are presented on Fig. 2E–H.

At low molar ratio, $R = 0.5$, the binding fast and almost mono exponential. The rate is mostly determined by the diffusion and

Table 1
The mean values and standard deviations of the equilibrium (K_1 & K_2) and kinetic (k_{+1}, k_{+2}, k_{-1} & k_{-2}) constants obtained in the result of the fitting of kinetics curves obtained at various S1/actin ratios. Fl_1, Fl_2 and Fl_3, which are values of fluorescence signal for actin alone (it equals to 1), respectively, were used as variable parameters with fixed limits.

$K_1 \times 10^6$, M^{-1}	28.9 ± 1.5
$k_{+1} \times 10^6$, $M^{-1}s^{-1}$	27.3 ± 2.6
k_{-1}, s	0.9 ± 0.1
K_2	3.3 ± 0.2
k_{+2}, s^{-1}	856.3 ± 39.5
k_{-2}, s^{-1}	261.4 ± 9.5
Fl_0, a.u.	0.99 ± 0.03
Fl_1, a.u.	0.70 ± 0.03
Fl_2, a.u.	0.34 ± 0.01

binding of S1 to actin in state 1 followed by rapid transition to state 2. At high molar ratio, $R = 4.0$, the binding is also fast and almost monophasic: S1 molecules rapidly occupied all actin sites predominately in state 1 since transition to state 2 is restricted by high density of S1 on actin filament. At intermediate ratios, $0.5 < R < 2.0$, we observed a bi-phasic kinetics: initial rapid change in signal followed by slow change. Our model provides the explanation of this slow phase of kinetics as a replacement of some S1 in state 2 by S1 in state 1. The S1s that arrived first to the actin would quickly occupy 2 actin monomers per S1, however, later they would be competitively transformed to state 1 by other S1 to reach a thermodynamic equilibrium. The slower rate of phase II is determined by growing steric restriction for binding of S1 and re-distribution of bound S1 between state 1 and state 2. The simulation curve for m2 concentration showed that it initially increases and then decreases, which means that at the beginning the probability to bind 2 actins was high but later it became less and an equilibrium between state 1 and state 2 shifted towards state 1 at higher R values (>1.0). In kinetics studies carried out and published previously, most experiments were done at high molar ratio of S1 to actin and fitted by simple Michaelian equation [15,16]. At these conditions a contribution of state 2 complexes was not significant and association constant between S1 and F-actin is lower than that at low ratios ($R < 0.5$). There were several

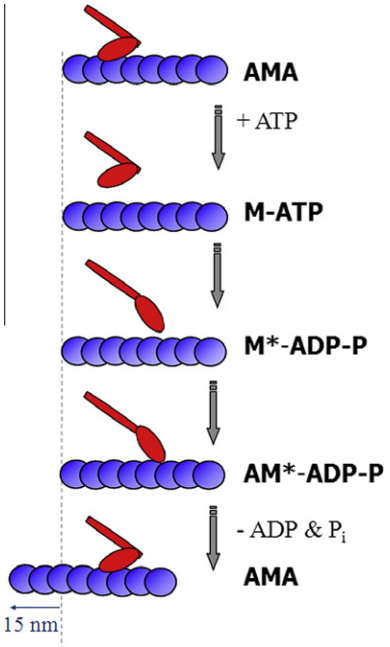

Fig. 4. Schematic presentation of the mechanism of force general and muscle contraction. Myosin head is bound to two actins, binding of ATP dissociates myosin from actin filament, following ATP hydrolysis is accompanied with unbending and extension of myosin (it is important to note that this step is occurred in absence of any mechanical load and energy loss), myosin binds to one actin (state 1) and then to two actins (state 2). Total sliding distance per one cycle is about 15 nm.

reports about observed negative cooperativity of S1 binding to F-actin, however the origin of anti-cooperativity was not identified. Blanchoin et al. [10] obtained experimental data similar to ours but they fitted their data using an artificial assumption that association constant decreased exponentially with increase of occupancy of F-actin. Despite that this assumption gave relatively good fit to experimental data it did not provide a molecular mechanism of anti-cooperativity. Our model based on experimental evidence that S1 can form two different complexes with stoichiometry 1:1 or 1:2 (S1: actin ratio). Kinetics of binding showed a strong dependence on the molar ratio of S1/actin, which can be explained by our model. Ability of myosin head to bind in sequence, initially, one actin and, then, two actins can have a fundamental significance since the transition from state 1 to state 2 might be associated with force generation and directed movement. Schematic presentation of a possible mechanism of force generation and muscle contraction is shown on Fig. 4. In our previous work we showed that S1 indeed first binds one actin via loop 627–637 and then binds the second actin via loop 567–574 [13]. Such transition might provide about 10 nm (the size of two actins) sliding distance in muscle fiber. ATP binding and hydrolysis induce dissociation of myosin head from actin, followed by its unbending [17] and re-orientation for the binding to the next site further from the previous one, which can provide an additional 5 nm to total distance per myosin step.

Acknowledgments

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