TWO-STEP LIGAND BINDING AND COOPERATIVITY

A Model to Describe the Cooperative Binding of Myosin Subfragment 1 to Regulated Actin

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ABSTRACT The binding of actin to myosin subfragment 1 (S1) has been shown to occur as a two-step reaction. In the first step actin is weakly bound and then the complex isomerizes to the "rigor type" acto–S1 complex (Coates, J. H., A. H. Criddle, and M. A. Geeves, 1985 *Biochem. J.*, 232:351–356). We propose here a model in which troponin/tropomyosin (Tn/Tm) controls the actin–S1 interaction by inhibiting the isomerization step. In this model the $(actin)_7$ Tn/Tm unit is assumed to exist in two states: open and closed. S1 can bind to either of the two states but only the open form allows the isomerization reaction to take place. We demonstrate that this model can account for the cooperative binding of S1 and S1 nucleotide complexes to actin. The model provides a way of integrating both the effects of calcium and nucleotide on actin–S1 interactions.

INTRODUCTION

The steric blocking model is the widely accepted view of the control of muscle activation by calcium. In this model (1–3) the troponin/tropomyosin (Tn/Tm) complex, in the absence of calcium, blocks the myosin binding site on seven actin monomers in the thin filament. Calcium binding to troponin causes a conformational change in the Tn/Tm complex, which allows access of myosin to actin. This model is compatible with recent time-resolved x-ray studies on whole muscle (4). These studies have been interpreted as showing that the first major structural event that occurs upon stimulation of a muscle is the change in the position of tropomyosin on the actin filament. This precedes both the movement of myosin heads towards the thin filament and the development of tension in the muscle. However, these studies do not define the position of the tropomyosin molecule on the thin filament in relation to the myosin binding site of actin. Recent evidence from both solution and skinned fiber studies (5–7) suggests that myosin can bind weakly to the thin filament even in the blocked or switched off state, which is not compatible with the simple steric blocking model. The interpretation of the solution studies led to the proposal that Tn/Tm inhibits the actomyosin ATPase by inhibiting an isomerization of the actomyosin products complex which controls the rate of phosphate release.

Greene and her collaborators have made a detailed study of the binding of myosin subfragment 1 (S1) to regulated actin using sedimentation methods (8, 9). They have shown that the binding of S1 to regulated actin exhibits positive cooperativity and have interpreted their results in terms of a cooperative model in which the actin filament is assumed to exist in two forms, a weak and a strong S1 binding form (10–12). The cooperative unit is proposed to be the seven actin monomers bridged by a single tropomyosin (A_7 Tn·Tm).[1] We propose here a model that is a development of the ideas proposed by Greene and her colleagues using some recent kinetic data on the interaction between actin and S1. The model predicts binding curves very similar to those of the Greene model and it suggests a molecular mechanism that can account for the effects of both calcium and nucleotide on the actin–S1 interaction. We present here a detailed formulation of the model and show how it can be tested against published data on the binding of S1 to regulated actin.

BACKGROUND

The work presented here has its origins in the model proposed by Geeves et al. (14) for the binding of S1 and S1·nucleotide complexes to actin in the absence of Tm/Tn. Essentially the model proposed that the binding of myosin or a myosin nucleotide complex to actin takes place

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[1]More recently Hill et al. (1980) have extended this model to allow for additional cooperativity between A_7 Tn·Tm units in the actin filament. We will limit the discussion in the present paper to models in which only the first type of cooperativity is effective while recognizing that a complete description of the binding of S1 to actin may have to include this secondary level of cooperative interaction.

in two major steps:

$$A + M\cdot N \overset{K_a}{\rightleftharpoons} A\text{–}M\cdot N \overset{K_b}{\rightleftharpoons} A\cdot M\cdot N.$$

In the first step myosin binds relatively weakly to actin with a binding constant (K_a) of $\simeq 10^4$ M^{-1} (0.1 M KCl, 20°C, pH 7). In the second step the complex can isomerize to the rigor-like complex but the extent to which the isomerization takes place is dependent upon the nature of the occupancy of the myosin–nucleotide binding site. In the absence of nucleotide, or when ADP is bound, then $K_b \gg 1$ and the rigor-like complex predominates ($A\cdot M\cdot N$). When ATP, or both products ($ADP\cdot P_i$) are bound, then $K_b \ll 1$ and the weakly attached state ($A\text{–}M\cdot N$) predominates. Recent results from this laboratory have provided experimental evidence that supports this model. Coates et al. (15) have shown that the presence of a pyrene label on cys 374 of actin reports the isomerization step of the binding reaction specifically. This allowed the measurement of both K_a and K_b in the absence of nucleotide (5.9×10^4 M^{-1}, 280 respectively; pH 7.0, 0.1 M KCl, 20°C). Using the same labeled actin Geeves et al. (16) demonstrated that at low ionic strength $K_b < 10^{-3}$ in the presence of ATP. Geeves and Halsall (17) have shown that the same model is valid for the binding of S1 to regulated actin in the presence of calcium and absence of nucleotide. The presence of Tm/Tn and calcium had no effect on K_b but did increase the affinity of S1 for actin by a factor of 7 by increasing K_a. This could be due to either a direct interaction between S1 and tropomyosin in the weakly bound state or to tropomyosin stabilizing the S1 binding site.

In the discussion of the two-step model of S1 binding to actin Geeves et al. (14) suggested that tropomyosin could control actomyosin interactions by a calcium-dependent inhibition of the isomerization step. This would result in only those myosin nucleotide complexes for which $K_b > 1$ being affected by calcium as was observed by Chalovich and Eisenberg (7). A feature of this model is that if K_a is unaffected by the presence of nucleotide then nucleotide will bind with the same affinity to free S1 as to S1 in the weak complex (A–M). This implies that there is no activation of the product release steps by actin binding in the weak state as required by the model. Therefore both force generation and activation of the ATPase are only achieved on attaining the strongly attached state, and tropomyosin can inhibit both of these activities by inhibiting the weak to strong transition.

THE MODEL

The model makes the following assumptions: (*a*) The cooperative unit is the seven actin monomers bridged by a single tropomyosin. (*b*) This A_7 unit exists as a dynamic equilibrium between an open and a closed state where the equilibrium constant K_T is defined as

$$K_T = [A_7]^{open}/[A_7]^{closed}.$$

(*c*) Each actin monomer acts independently in binding S1, i.e., the affinity of actin for S1 is independent of the presence of S1 on adjacent actin monomers. (*d*) S1 binds to actin in two steps as proposed by Geeves et al. and that binding in the first step is independent of the state of the A_7 unit. If ○ represents an actin monomer in the closed state and □ a monomer in the open state then for the binding of a ligand × the equilibrium constant K_a is defined as

$$K_a = \frac{[\boxtimes]}{[\square]\,[\times]} = \frac{[\otimes]}{[\mathrm{O}]\,[\times]}.$$

(*e*) Only those monomers in the open state allow the second step of the S1 binding reaction, the isomerization defined by K_b in the first scheme. If the isomerization step is represented by

$$\boxtimes \rightleftharpoons \boxdot \text{ and } K_b = \frac{[\boxdot]}{[\boxtimes]},$$

then the overall scheme can be represented as shown in Fig. 1.

The equations to describe the model in Fig. 1 will be discussed in terms of a four-site cooperative unit as an illustration with the equations generalized to p ligand binding sites presented at the end.

With K_a, K_b, and K_T defined as above, the concentration of each species in Fig. 1 can be defined in terms of the equilibrium constants, the concentration of free ligand, and the concentration of open actin units with no bound ligands ($\square\square\square\square$), e.g.,

$$[\square\square\square\boxtimes] = 4K_a[\times][\square\square\square\square]$$

$$[\square\boxtimes\boxtimes\boxtimes] = \frac{4.3.2}{3.2}K_a^3[\times]^3[\square\square\square\square]$$

$$[\mathrm{O}\otimes\otimes\otimes] = K_T^{-1}[\square\boxtimes\boxtimes\boxtimes]$$

$$[\boxdot\boxdot\boxtimes\boxtimes] = \frac{4.3}{2}K_b^2[\boxtimes\boxtimes\boxtimes\boxtimes]$$

$$= \frac{4.3}{2}K_b^2\frac{4.3.2.1}{4.3.2.1}K_a^4[\times]^4[\square\square\square\square].$$

Using these defined concentrations it can be shown that the concentration of cooperative units with no bound ligand is given by

$$[\square\square\square\square] + [\mathrm{OOOO}] = [\square\square\square\square]\,(1 + K_T^{-1})$$

cooperative units with one site occupied

$$[\square\square\boxtimes\square] + [\otimes\mathrm{OOO}] + [\square\square\square\boxdot]$$
$$= [\square\square\square\square]\,4\,K_a[\times]\,(1 + K_T^{-1} + K_b)$$

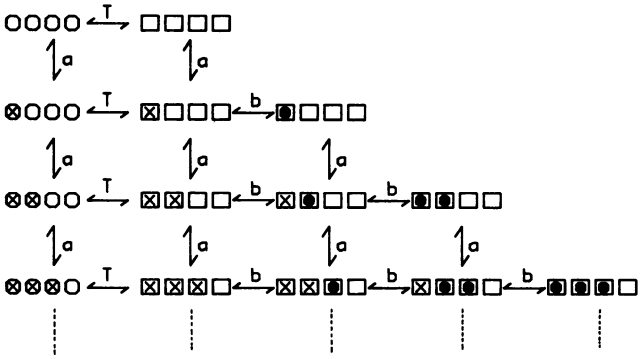

FIGURE 1 Diagrammatic representation of the two-step binding of a ligand to a four-site cooperative unit. Symbols are as defined in the text. Step *a* represents the weak binding of a ligand to a single site within the cooperative unit, step *b* is the isomerization of this weakly bound ligand to form the strongly bound complex, and step *T* is the isomerization between open and closed cooperative units.

with two, three, and four sites occupied by

Units with two occupied sites

$$= [\square\square\square\square]\frac{4.3}{2}K_a^2[\times]^2(1 + K_T^{-1} + 2K_b + K_b^2)$$

Units with three occupied sites

$$= [\square\square\square\square]\frac{4.3.2}{3.2}K_a^3[\times]^3\left(1 + K_T^{-1} + 3K_b + \frac{3.2}{3}K_b^2 + K_b^3\right)$$

Units with four occupied sites $= [\square\square\square\square]\dfrac{4.3.2.1}{4.3.2.1}$

$$\cdot K_a^4[\times]^4\left(1 + K_T^{-1} + 4K_b + \frac{4.3}{2}K_b^2 + \frac{4 + 3.2}{3 + 2}K_b^3 + K_b^4\right).$$

Thus the total concentration of sites is given by

$$\text{Total sites} = [\square\square\square\square]\,p\sum_{n=0}^{p}\frac{p!}{n!(p-n)!}(K_a[\times])^n \cdot \left(K_T^{-1} + \sum_{m=0}^{n}K_b^m\frac{n!}{m!\,(n-m)!}\right)$$

and the concentration of occupied sites by

$$\text{Occupied sites} = [\square\square\square\square]\sum_{n=0}^{p}\frac{n\cdot p!}{n!\,(p-n)!} \cdot (K_a[\times])^n\left(K_T^{-1} + \sum_{m=0}^{n}K_b^m\frac{n!}{m!\,(n-m)!}\right),$$

where p is the number of ligand binding sites in the cooperative unit. Thus the fractional saturation (θ) can be defined in terms of the three equilibrium constants and the free ligand concentration.

In terms of this model the system can show cooperative behavior if both K_b and K_T^{-1} are greater than 1 and the extent of cooperativity will depend upon the relative values of K_b and K_T. At low levels of saturation the affinity of ligand approaches

$$K_a\,[1 + K_b/(1 + K_T^{-1})]$$

and at high levels of saturation

$$K_a\,[1 + K_b/(1 + K_T^{-1})^{1/p}]\,.$$

These features of the model can be illustrated by simulations of the fractional saturations of actin by S1 as a function of free S1 concentration. Fig. 2 shows simulations of the model with values of K_T between 1 and 10^{-3}. K_a and K_b have been assigned the values defined by Geeves and Halsall (17) for the association of regulated actin and S1 in the absence of nucleotide at pH 7.5 and 0.1 M KCl (4×10^5 M^{-1} and 200, respectively). These simulations show that for any value of $K_T < 1$ the system exhibits cooperative binding and this behavior becomes more marked as K_T decreases. In this model it appears reasonable that the value of K_T would depend upon the amount of calcium bound to troponin, K_T being greater in the presence of calcium. Increasing concentrations of calcium would then shift the binding curve towards the noncooperative form. The presence of saturating calcium need not induce complete noncooperative behavior of the filament. Effective control of the interaction could be achieved by changing K_T in the range 10^{-1}–10^{-2}. Indeed the work of Greene on the binding of S1 to regulated actin in the presence of ADP (8) suggests that the binding is cooperative both in the presence and the absence of calcium. More recently Rosenfeld and Taylor (20) have examined the rate of calcium binding to thin filaments and they also interpreted their data in terms of a two-state model of the thin filament. Their data suggest that K_T (as defined here) is $\ll 1$ in the absence of calcium. Calcium binding then causes a 20-fold increase in K_T but its value still remains <1. The model presented here is compatible with these figures.

Fig. 3 shows simulations of the model with K_a and K_T held constant at 4×10^5 M^{-1} and 0.02, respectively, varying K_b over the range 0–1,000. Geeves et al. (14) proposed that K_b would vary over the range 10^{-3}–10^{+3}

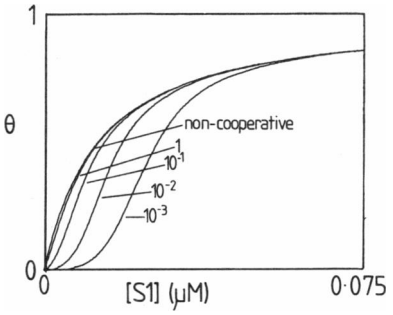

FIGURE 2 Simulations of the dependence of fractional saturation of sites θ on free ligand concentration at different values of the equilibrium constant controlling the ratio of open to closed cooperative units (K_T). The simulation assumes a seven-site cooperative unit, and $K_a = 4 \times 10^5$ M^{-1} and $K_b = 200$ labels denote values of K_T.

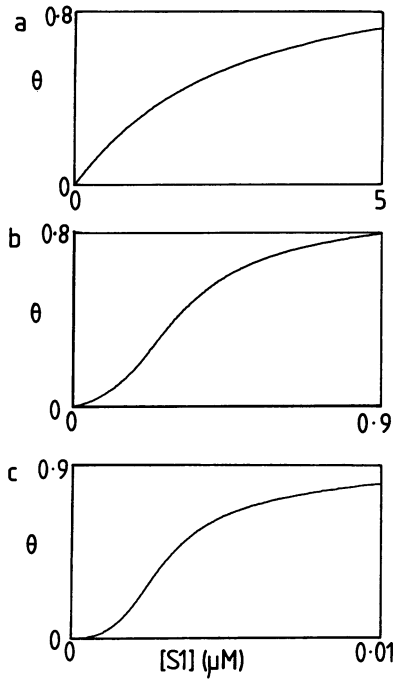

FIGURE 3 Simulations of the dependence of the fractional saturation of sites (θ) on free ligand concentration at different values of the tight binding isomerization equilibrium constant K_b for a seven-site cooperative unit. For each curve $K_a = 4 \times 10^5\ M^{-1}$, $K_T = 0.02$, and K_b is (*a*) 1, (*b*) 10, or (*c*) 1,000.

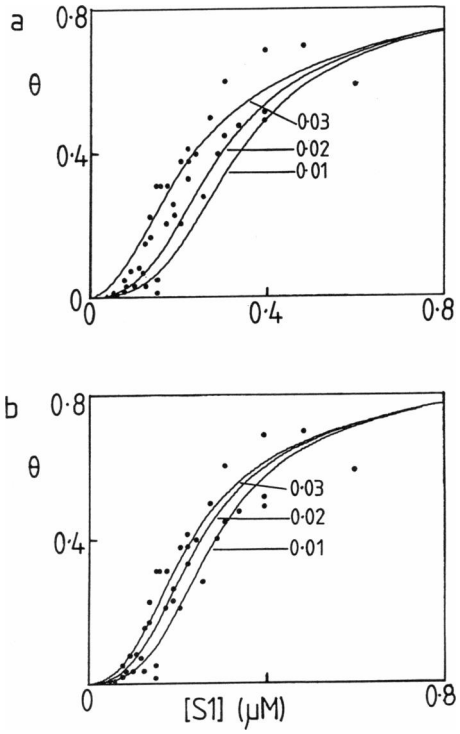

FIGURE 4 Simulations of the dependence of fractional saturation of sites (θ) on free ligand concentration for a seven-site model superimposed on the binding data of Greene (8) for S1 binding to regulated actin in the absence of calcium. For each curve $K_a = 3.3 \times 10^4\ M^{-1}$, and K_b is (*a*) 110 or (*b*) 130. Labels indicate the values of K_T. The data were obtained in 0.3 M KCl, 0.5 mM dithiothreitol, 1 mM $MgCl_2$, 5 mM KP_i, 15 mM imidazole, pH 7, 25°C.

depending upon the nucleotide bound to S1. These simulations show that provided that K_b is >10 the cooperative nature of the reaction is relatively insensitive to the value of K_b. For values of K_b <10 the cooperativity is reduced and for values <1 no cooperativity is apparent. However, as the overall binding constant varies, the free S1 concentration range over which the cooperativity can be observed is very different for the differing values of K_b.

The model as described here is essentially the same as that proposed by Greene and Eisenberg except that the binding of S1 to the weak and strong forms of the thin filament are defined by their model as K_w and K_s and in our model by K_a and K_a $(1 + K_b)$. The two models therefore predict very similar S1 binding isotherms but the model presented here provides a simple unifying theme to integrate both the effects of nucleotide and calcium on actin–S1 interactions. In this model estimates of K_a and K_b can be provided by kinetic measurements that are independent of the regulatory model. This leaves only K_T undefined, and the value of K_T is insensitive to the nucleotide bound to S1. Our recent kinetic experiments have provided estimates of K_a and K_b of $3.3 \times 10^4\ M^{-1}$ and 112 in the absence of nucleotide at 0.3 M KCl, pH 7.5, 20°C. These conditions are very similar to the conditions used by Greene to define the binding of S1 to regulated actin in the absence of both nucleotide and calcium. Fig. 4 *a* shows Greene's binding data superimposed on simulations of the model using our estimates of K_a and K_b and varying K_T in the range 0.01–0.03. As can be seen from the data a reasonable fit can be obtained with a value of K_T in this range. The best fit was achieved with the same value for K_a, $K_b = 130$ and $K_T = 0.02$ (Fig. 4 *b*). As Greene suggested with her data, a good test of a model is if the value of K_T obtained can be used to fit binding data in the presence of nucleotide. Greene used data under the same experimental conditions but in the presence of 3 mM ADP as a test of her model. We have not measured K_a and K_b in the presence of ADP but in the model of Geeves et al. it was suggested that the value of K_a would be relatively independent of the presence of nucleotide and that in the presence of ADP K_b would be ~10. Using these values a reasonable agreement with Greene's binding data in the presence of ADP was achieved (Fig. 5 *a*). Once again a reasonable fit can be achieved with $K_T = 0.01$–0.03. As stated above the model is not sensitive to the value of K_b provided that it is >10. This is demonstrated in Fig. 5 *b* where K_T is held constant at 0.02 in the term K_a $(1 + K_b)$ is constant at 3.63×10^5 M^{-1} while K_b is varied in the range 0–1,000.

We have shown here that the model proposed can predict the binding of S1 to actin at least as well as the normal cooperative model used by Greene. The main advantage of our model is that it integrates the rate and equilibrium data on the binding of S1 to unregulated actin

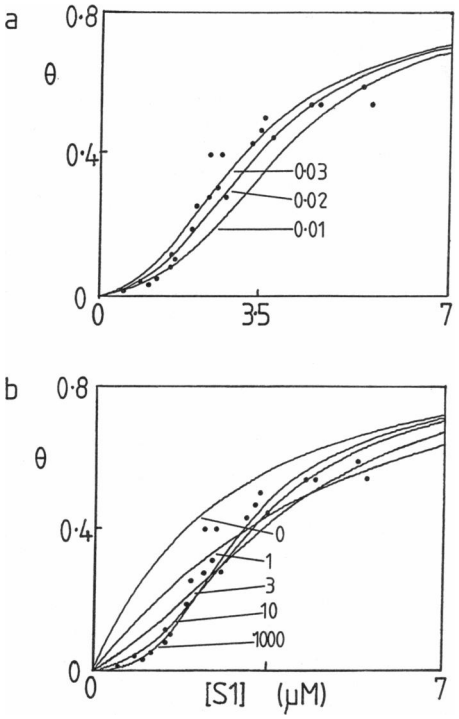

FIGURE 5 Simulations of the dependence of fractional saturation of sites (θ) on free S1 concentration superimposed on the data of Greene (8) in the presence of ADP. (*a*) $K_a = 3.3 \times 10^4\ M^{-1}$, $K_b = 10$, labels refer to values of K_T. (*b*) $K_T = 0.02$, $K_a(1 + K_b) = 3.63 \times 10^5\ M^{-1}$, and K_b varied as labeled. Experimental data were obtained under the same conditions as Fig. 4 except that 5 mM $MgCl_2$ and 3 mM ADP replaced 1 mM $MgCl_2$ and 5 mM KP_i.

in the presence of nucleotides with the cooperative binding to regulated actin. The model is compatible with the time-resolved x-ray studies of Huxley and co-workers (2) and the solution studies of Chalovich and Eisenberg (7). The model suggests weak attachment of crossbridges to give the A–M·N state will occur in both relaxed and active muscle but that calcium must bind to the Tm/Tn complex before the strongly attached A·M·N can be achieved. Attaining the A·M·N state results in an increased attachment of crossbridges, an increased rate of product release, and force generation. Therefore the binding of calcium and any subsequent change in the structure of the Tm/Tn complex must precede both the increase in the number of attached bridges and force generation which occur on activation. In solution, when ATP is bound to S1, $K_b < 10^{-3}$ and no effect of calcium upon actin binding will be apparent. Chalovich and Eisenberg (7) proposed that Tn/Tm could control the ATPase by controlling the rate of P_i release rather than by controlling actin binding, as proposed in the original steric blocking model. Our view is essentially similar to this except we identify the regulated step as the specific isomerization of the acto·S1 nucleotide complex. In the case of the steady-state products complex the step is

$$\text{A–M·D·P} \overset{K_b}{\rightleftharpoons} \text{A·M·D·P}.$$

Little direct information is available on this step but studies with nucleotide analogues suggest a rapid equilibration step with $K_b \approx 0.1$. Subsequent release of P_i provides the necessary free energy to drive the reaction to completion.

The kinetics of the interaction between actin and S1 predicted by this model are also of interest. Trybus and Taylor (18) studied the kinetics of S1 binding to regulated actin in the absence of calcium and demonstrated that the reaction showed a lag phase that could be eliminated by preincubating the actin with substoichiometric amounts of S1. They concluded that a model of the type used by Greene and Eisenberg could not account for their results as these suggested that more actin sites were available for S1 to bind to than prediced by the model. The alternative that they considered was that tropomyosin is not a rigid rod but is flexible enough to allow some sites within the A_7 unit to be open and some closed at least transiently. (A more detailed alternative interpretation of their data was proposed by Balzacs and Epstein [19].)

In the model proposed here all of the actin sites are available to S1 at any time but only weak binding takes place unless the cooperative unit is in the open conformation. The rate of S1 binding will be controlled primarily by k_{+a} (where $K_a = k_{+a}/k_{-a}$) and the amplitude and presence of any lag phase will be controlled by the relative values of K_T and K_b and by the rates k_{+T}. The rate $k_{+b} + k_{-b}$ has been shown to be very fast (>4,000 s^{-1} [17]). Detailed kinetic simulations will be required before the kinetics of this system can be adequately assessed in relation to the studies of Trybus and Taylor (18). Indeed a detailed kinetic study will provide the best test of the proposed model.

It has not escaped our attention that the model proposed here for actin–myosin interactions may be appropriate for other cooperative systems. An essential feature of the model is that if K_a and K_b can be defined under conditions where the system behaves in a noncooperative manner then it becomes a relatively simple task to define K_T from the binding isotherm under cooperative conditions. Similarly if K_T can be defined from some intrinsic physiochemical property of the protein then K_a and K_b can readily be obtained from the ligand binding isotherm.

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