Theoretical Kinetic Studies of Models for Binding Myosin Subfragment-1 to Regulated Actin: Hill Model versus Geeves Model

Yi-der Chen,* Bo Yan,* Joseph M. Chalovich,† and Bernhard Brenner‡

*Mathematical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, 9190 Rockville Pike, Bethesda, Maryland 20892-2690, †Department of Biochemistry, East Carolina University Medical School, Greenville, North Carolina 27858-4354 USA and ‡Department of Molecular and Cell Physiology, Medical School Hannover, D-30623 Hannover, Germany

ABSTRACT It was previously shown that a one-dimensional Ising model could successfully simulate the equilibrium binding of myosin S1 to regulated actin filaments (T. L. Hill, E. Eisenberg and L. Greene, Proc. Natl. Acad. Sci. U.S.A. 77:3186–3190, 1980). However, the time course of myosin S1 binding to regulated actin was thought to be incompatible with this model, and a three-state model was subsequently developed (D. F. McKillop and M. A. Geeves, Biophys. J. 65:693–701, 1993). A quantitative analysis of the predicted time course of myosin S1 binding to regulated actin, however, was never done for either model. Here we present the procedure for the theoretical evaluation of the time course of myosin S1 binding for both models and then show that 1) the Hill model can predict the “lag” in the binding of myosin S1 to regulated actin that is observed in the absence of Ca$^{++}$ when S1 is in excess of actin, and 2) both models generate very similar families of binding curves when [S1]/[actin] is varied. This result shows that, just based on the equilibrium and pre-steady-state kinetic binding data alone, it is not possible to differentiate between the two models. Thus, the model of Hill et al. cannot be ruled out on the basis of existing pre-steady-state and equilibrium binding data. Physical mechanisms underlying the generation of the lag in the Hill model are discussed.

INTRODUCTION

Muscle contraction involves cyclic binding and unbinding of myosin heads (cross-bridges) of the thick filament to actin of the thin filament driven by the hydrolysis of ATP. Tropomyosin (Tm) and troponin (Tn) are proteins responsible for the Ca$^{++}$ regulation of the interaction between myosin and actin in vertebrate skeletal muscle. In solution, myosin and its subfragment 1 (S1) bind to unregulated filamentous actin (F-actin in the absence of Tm and Tn) with no measurable cooperativity, and the binding kinetics may involve at least two binding steps (Trybus and Taylor, 1980; Geeves and Halsall, 1987). In the presence of Tm and Tn, the equilibrium binding is highly cooperative in the absence of Ca$^{++}$ and is slightly cooperative in the presence of Ca$^{++}$ (Greene and Eisenberg, 1980). The cooperative binding isotherm at equilibrium was first interpreted with a one-dimensional Ising model by Hill et al. (1980) (referred to as the Hill model from now on). In this model (see Fig. 1), a single Tm–Tn and seven actin monomers form a unit that can exist in two states, state 1 (inactivated) and state 2 (activated), with higher binding affinities for Ca$^{++}$ and myosin (or S1) for the latter. Thus, the binding of Ca$^{++}$ and myosin to the actin modulates the distribution between the two states of each unit in the thin filament. The binding of myosin is cooperative because seven actin monomers in a unit change state as a group and because of the existence of interactions between two neighboring units. Because equilibrium binding was the only concern, the kinetics of the binding reactions was not specified in the model. The Hill model, however, was not only shown to account for equilibrium binding data but also for the steady-state kinetic patterns of ATP hydrolysis at both high and low free Ca$^{++}$ and for both excess S1 and excess actin (Hill et al., 1981). This model also incorporated the different effect of tropomyosin–tropomyosin on the binding of different nucleotide complexes of S1 (Chalovich et al., 1981).

The cooperative S1 binding was reinterpreted by Geeves and his colleagues in terms of a two-state model at first (Geeves and Halsall, 1987; McKillop and Geeves, 1991) and then a three-state model (McKillop and Geeves, 1993) (referred to as the Geeves model from now on), in which the S1 binding kinetics are strictly coupled to the two-step mechanism of S1 binding to F-actin in the absence of Tm and Tn. In this three-state model (see Figs. 2 and 3), seven actin monomers and a Tm–Tn molecule also form a unit. Each unit in the thin filament can exist in three states with different S1 binding properties: the “blocked” state where S1 binding is completely prohibited, the “closed” state where only the first step of the two-step binding mechanism can take place, and the “open” state where both steps are in operation. In this model, the equilibrium association constant and the kinetic rate constants of binding of S1 to actin monomers in the three states are independent of the presence of Ca$^{++}$. Ca$^{++}$ affects only the distribution between the blocked and the closed states and therefore the availability of S1 binding sites on the actin filament. In addition, this model does not consider explicitly the nearest-neighbor interaction between cooperative units.
Recently, the molecular structure of regulated actin filaments has been studied using electron microscopy and three-dimensional image reconstruction (Vibert et al., 1997; Xu et al., 1999). It has been shown that addition of Ca\(^{2+}\) causes an \(\approx 25^\circ\) azimuthal movement of tropomyosin from the outer to inner domain of actin and that S1 binding causes a further 10° shift, resulting in complete exposure of the myosin binding site on actin. That is, structurally the actin filament can be considered to exist in three states: the fully-off, the intermediate calcium, and the fully-on state (Tobacman and Butters, 2000). It is easy to assume that the Geeves model is more consistent with this structural information, because it also contains three kinetic states. However, the Hill model is also consistent with this three-state structural model because the Hill model has enough sub-states to predict different levels of ATPase activity for the conditions used to obtain three structures of the actin filament (Hill et al., 1981). At low saturation with myosin and in the absence of Ca\(^{2+}\), the actin units are in state 1(0), where the subscript (0) means that the actin unit has no bound Ca\(^{2+}\). At low bound S1 and in the presence of saturating Ca\(^{2+}\), the rate is increased \(\sim\)80-fold (28-fold increase in \(k_{\text{cat}}\) and decrease in the \(K_M\) to 0.3 of the original value; Chalovich and Eisenberg, 1982). At this condition, actin filaments are in state 1(2) of the Hill model (the actin filament is in the same major state 1, but it is in a different sub-state with two bound Ca\(^{2+}\) ions per troponin). Finally, at high saturation with myosin and with bound Ca\(^{2+}\), the actin filament is in state 2(2) and the rate increases another eight-fold (two-fold increase in \(k_{\text{cat}}\) and a decrease in the \(K_M\) to 0.25; Williams et al., 1988). It is thought that the ability of actin to stimulate the ATPase activity of myosin is about the same if actin is in state 2(0) (no bound Ca\(^{2+}\)) and in state 2(2) (two bound Ca\(^{2+}\)). Thus, there are sufficient chemical states in both the Hill model and the Geeves model to account for the observed structural states. A major difference between the two models is that the intermediate structural state is assumed to be required in the absence of Ca\(^{2+}\) in the Geeves model, but not in the Hill model.

As far as fitting the equilibrium binding isotherms for S1 to actin is concerned, the Hill and Geeves models are mathematically equivalent. However, the molecular kinetic
mechanisms of activation and myosin binding of the two models are fundamentally different. For example, in the Geeves model the rate constant of binding of S1 to actin monomers in a regulated actin filament is independent of whether the troponin of the unit is bound with Ca\(^{2+}\) or not, but this is not true for the Hill model. Also, the two-step mechanism for the binding of S1 to an actin site in the open state. The same mechanism operates in the binding of S1 to pure F-actin in the absence of Tm–Tn. (C) The complete kinetic diagram for the binding of S1 to a Tm–Tn unit for the special two-actin case. The diagram can be extended to cases with an arbitrary number of actin monomers as shown in Fig. 3 for the seven-monomer case.

Recently, the pre-steady-state kinetics of S1 binding to regulated actin filaments in the absence and presence of Ca\(^{2+}\) have been studied in several laboratories by measuring light scattering and fluorescence changes in pyrene-labeled actin (Trybus and Taylor, 1980; McKillop and Geeves, 1993). It has been found that, when actin is in excess of S1, the time course of the fluorescence change or the light scattering can be described by a single exponential function in both the presence and absence of calcium. In contrast, when S1 is in excess of actin, a single-exponential is observed in the presence but not in the absence of Ca\(^{2+}\). In the absence of Ca\(^{2+}\), the shape of the time course of myosin S1 binding to regulated actin is sigmoidal (referred to as having a “lag”). It was assumed that the two-state kinetic mechanism of the Hill model would not be able to generate this kind of lag in the time course of S1 binding. Quantitatively, it was also argued that the Hill model did not have enough actin in the on state before S1 mixing to account for the fast S1 binding rate measured at early times (Trybus and Taylor, 1980). The three-state Geeves model was assumed to be able to solve all these problems (McKillop and Geeves, 1993). However, a quantitative analysis of the entire time course of S1 binding to regulated actin predicted by the two models has never been carried out; the properties of the models have only been discussed qualita-
tively based on the apparent rate constants obtained by fitting the time courses with a one-exponential function (McKillop and Geeves, 1993). Thus, it is not clear whether the Hill model is really unable to generate the observed lag and the fast initial rate in the kinetic curves of S1 binding. Nor has it been clearly demonstrated that the Geeves model is really able to predict the characteristic family of time courses of S1 binding for different S1 and actin concentrations.

In this paper, the kinetic properties of the two models are studied theoretically with the aim to address the questions mentioned above. We first discuss the mathematical procedures for the calculation of time course for S1 binding and then discuss the predictions of the two models. Due to the explicit inclusion of cooperativity in the model, the kinetics of S1 binding in the Hill model has to be evaluated using the Monte Carlo simulation method. In contrast, the kinetic binding curves of the Geeves model can be calculated numerically. We show that both models are equivalent in predicting the kinetics of S1 binding to regulated actin. Specifically, we show that, in contrast to what was suggested in the past, the Hill model is just as well able to generate the lag in the time course of S1 binding to regulated actin when excess S1 is mixed with actin in the absence of calcium. This shows that it is not possible to differentiate the two models based on equilibrium and pre-steady-state kinetic data of S1 binding alone. This result also implies that, in the absence of Ca\(^{2+}\), the activation of actin filament from the off to the on state does not require the existence of an intermediate state as in the Geeves model. The mechanisms underlying the generation of the lag in the S1 binding kinetic curves in the two-state model of Hill are discussed.

We have not attempted to evaluate all possible models of regulation but have focused on the original Hill model and the Geeves three-state model with the intent of understanding those factors responsible for the complex binding and kinetics of the regulated actomyosin system. Several other theoretical treatments of regulation have been published such as the elaborate models of Zou and Phillips (1994) and Tobacman and Butters (2000).

**MODEL AND MATHEMATICAL ANALYSIS**

**The Hill model**

The basic equilibrium and kinetic features of the Hill model are described schematically in Fig. 1. A regulated actin filament is composed of a linear array of units, each of them contains a Tm–Tn complex and 7 actin monomers. Each unit can exist in two states, state 1 (the inactive, or off, state) and state 2 (the active, or on, state), with different Ca\(^{2+}\) and S1 affinities. Let \(w_{ij}\) (\(i, j = 1, 2\)) denote the interaction energies between two neighboring units in states \(i\) and \(j\), respectively, as shown in Fig. 1A. And, as shown in Fig. 1B, let \(K_1\) and \(K_2\) denote the equilibrium constants of

---

**FIGURE 3** The complete kinetic diagram of the Geeves model for the binding of S1 to a Tm–Tn unit with seven actin monomers. The configurations of some states are shown at the upper right corner. The dotted block arrow represents the transition from the blocked to the closed state and the blank block arrow represents the transition from the closed to the open state. Arrows with barbed ends and those with triangle ends represent, respectively, the first and the second steps of the two-step S1 binding reactions.

Biophysical Journal 80(5) 2338–2349
binding S1 to an actin monomer in states 1 and 2, respectively, and L be the intrinsic equilibrium constant for the transition from state 2 to state 1 of an isolated Tm–Tn–actin unit with no bound S1. Then, as shown by Hill et al. (1980), the fraction of the total actin monomers bound with S1 (θ) in this model can be expressed as a function of the concentration (c) of the free S1 in solution as

\[ \theta = p_1 \frac{K_c}{1 + K_c c} + p_2 \frac{K_c}{1 + K_c c}, \]

where

\[ p_1 = \frac{2aY^{-1}}{\sqrt{(1 - a + \sqrt{1 - a})}}, \quad p_2 = \frac{2aY^{-1}}{\sqrt{(1 + a + \sqrt{1 + a})}}, \]

\[ Y = \frac{Y_1 Y_2 Y_1^2}{L'}, \]

and \( Y_i = \exp(-\omega_i/k_B T) \), where \( \omega_i \) is the interaction energy between two neighboring units in states i and j, and \( k_B T \) is the product of the Boltzmann constant and the temperature (the cooperativity factor). For simplicity, we assume that \( Y_1 = Y_2 \), Eq. 1 can thus be used to evaluate the values of \( K_1, K_2, L' \), and Y from the experimental binding isotherm.

One must note that Eq. 1 defines the fraction of the total actin monomers bound with S1. Thus, Eq. 1 can be used directly for data analysis when the measured quantity reflects the total amount of S1 bound to actin (such as when measuring the light scattering). Recently, the fluorescence of pyrene attached to actin monomers has been shown to monitor the amount of actin monomers in state 2 that are bound with S1. In this case, the fractional change of the pyrene fluorescence in the system, \( R(c) \), is related to the second term of Eq. 1 as

\[ R(c) = \frac{F(c) - F(0)}{F(\infty) - F(0)} = p_2 \frac{K_c}{1 + K_c c}, \]

where \( F(\infty) \) represents the total pyrene fluorescence of the system when the free S1 concentration in the system is c.

Due to the existence of cooperativity, the kinetic curves of S1 binding to actin filaments have to be evaluated by Monte Carlo simulation. The basic rate constants describing the kinetics of S1 binding and conformational transitions of an isolated Tm–Tn–actin unit are shown in Fig. 1B. They are the S1 binding and dissociation rate constants, \( k_1, k_0, k_2, k_d, \) and the transition rate constants of an isolated Tm–Tn–actin unit between active (state 2) and inactive (state 1) states, \( \alpha_m \) and \( \beta_m (m = 0, 1, 2, \ldots, 7) \) where m refers to the number of S1 bound to the unit. These rate constants are not independent of each other, but related to the equilibrium constants,

\[ k_1/k_0 = K_1, \quad k_2/k_1 = K_2, \]

\[ \beta_d/\alpha_0 = L, \]

\[ \beta_m/\alpha_m = L(K_m/K_2)^m, \quad m = 1, 2, \ldots, 7. \]

Because \( K_1 \) and \( K_2 \) can be obtained from the equilibrium-binding isotherm, only \( \alpha_0 \) and \( \beta_m \) are independent parameters. In general, with a given L (the values of \( \alpha_0 \) and \( \beta_m \) are assigned) there are infinite ways to assign the values of individual \( \alpha_m \) and \( \beta_m \) that are consistent with Eq. 10. Depending on how the factor \( (K_i/K_j)^m \) is partitioned between the two rate constants. For simplicity, let us assume that they can be expressed as

\[ \alpha_m = \alpha_0 (K_i/K_j)^{(m-1)} \gamma \quad (11), \]

\[ \beta_m = \beta_0 (K_i/K_j)^{(m-1)}, \]

where \( \gamma \) is a parameter with values between 0 and 1. Note that Eqs. 11 and 12 are also applicable to the \( m = 0 \) case and therefore are the general rate expressions for the transitions between states 1 and 2 of a Tm–Tn–actin unit.

The kinetic parameters discussed above are for isolated Tm–Tn–actin units only. In a filament, the transition rate constants, \( \alpha_m \) and \( \beta_m (m = 0, 1, \ldots, 7) \), are influenced by the cooperativity of the system. Let \( \alpha_m \) and \( \beta_m \) represent the cooperativity-affected rate constants. Then, as shown in Appendix A, they can be expressed as

\[ \tilde{\alpha}_m = \alpha_0 (Y_1 Y_2)_{(N_2 - 1)/2} Y_2^{2(1 - \delta)} \]

\[ \tilde{\beta}_m = \beta_0 (Y_1 Y_2)^{(N_2 - 1)/2} Y_2^{2\delta}, \]

where \( \delta \) is a constant value between 0 and 1, \( Y_1 = Y_{1/2}/Y_{1/2}, \)

\( Y_2 = Y_{2/2}/Y_{1/2}, \)

\( N_2 = \) the number of the two neighboring units that are in state 1. Note that \( N_2 \) can have values of 0, 1, or 2 only (see Fig. 1C) and that the values of \( Y_1 \) and \( Y_2 \) can be evaluated from Eqs. 5 and 6 when the values of Y and \( L' \) are determined from equilibrium binding isotherm and the value of L is given by the assigned \( \alpha_0 \) and \( \beta_0 \). In fitting the model to any given kinetic curve, \( k_1, k_2, \alpha_0, \beta_0, \gamma, \) and \( \delta \) are chosen as the independent parameters.

To evaluate the kinetics of S1 binding using the Monte Carlo method, we follow the transitions of a single actin filament among its various states on a computer as a function of time and obtain the ensemble-averaged time course by repeating the process for a large number of times (Chen and Hill, 1983; Chalovich et al., 1995). That is, we treat the actual system as an ensemble of identical and independent small systems, each containing only one actin filament with a small volume. The kinetics of the actual system is then represented by the ensemble average of the kinetics of a single small system. During the simulation, the concentration of free S1 in solution changes as a function of time. Let \( c_0 \) and \( c_f \) be the total concentrations of S1 and actin monomers, respectively. Then the concentration of free S1 within each small system can be expressed as \( c = c_0 - (n/M) \alpha_{c, n} \) where \( n \) is the number of bound S1 on an actin filament and M is the total number of actin monomers in an actin filament (M = 700 in all calculations).

The program to simulate the experimental S1 binding process consists of two parts. At first, the system is simulated in the absence of S1 until the system is at equilibrium. Then S1 is added to the system and the kinetic simulation starts. Histograms of bound S1 in the two states are obtained as a function of time after S1 addition. The process is repeated a thousand times and the kinetic curve of S1 binding is obtained from the average of these histograms. In this study, the number of actin monomers in an actin filament is fixed at 700. Doubling the number to 1400 was found to cause very little change in the simulated results.

### The Geeves model

As in the Hill model, a regulated actin filament is also composed of Tm–Tn–actin units. Each unit contains seven actin monomers and can exist in three states: blocked, closed, and open (see Fig. 2A). A unit is unable to bind S1 if the unit is in the blocked state; S1 can only bind to the closed and open states. The binding of S1 to actin involves a two-step mechanism with the second step occurring only when the unit is in the open state (see Fig. 2B). In contrast to the Hill model, there is no cooperativity between neighboring units. Therefore, each unit can be treated independently. The diagram describing the kinetic mechanism of the model is shown in Fig.
Kinetics of Binding S1 to Regulated Actin

Let \( K_1^c \) and \( K_2^c \) be the equilibrium constants of the two-step S1 binding reactions to actin and \( K_a \) and \( K_T \) be the equilibrium constant between the blocked and the closed and between the closed and the open states, respectively. Then the fraction of total actin monomers bound with S1 can be expressed as (McKillop and Geeves, 1993)

\[
\theta = \frac{K_1^c c (1 + K_2^c c) P^6 + Q^7}{K_1^c P + Q^1 + 1/K_B^c},
\]

where \( P = 1 + K_2^c c (1 + K_2^c c), Q = 1 + K_1^c c, \) and \( c \) is the concentration of the free S1 in solution. In contrast, if one is measuring the fluorescence of pyrene attached to actin, then the fractional change in fluorescence is related to these constants as

\[
R(c) = \frac{K_1^c c P^6 (1 + K_2^c c) + 1}{(K_1^c P + Q^1 + 1/K_B^c)(1 + K_2^c c)^5}.
\]

Eqs. 15 and 16 can be used to evaluate the equilibrium constants, \( K_1^c, K_2^c, K_a, \) and \( K_T \) using the binding isotherm.

As shown in Fig. 2 C, there are eight basic rate constants for the Geeves model: \( \alpha_{AB}, \alpha_{A}, \alpha_{T}, \alpha_{B}, k_{1} - k_{2}, k_{3} - k_{4}, k_{3} \). However, they are related to the equilibrium constants as: \( K_B = \alpha_A / \alpha_B, K_T = \alpha_T / \alpha_B, K_1^c = k_{1} / k_{2}, K_2^c = k_{3} / k_{4}. \) Thus, only four rate constants are independent.

With the kinetic diagram given in Fig. 3, the differential equations describing the kinetic behavior of the probability, \( p_i(t) \), for each state in the diagram can be written down immediately. For example,

\[
\frac{dp_1(t)}{dt} = -\alpha_B p_1 + \alpha_{AB} p_2,
\]

\[
\frac{dp_2(t)}{dt} = \alpha_B p_1 - (\alpha_B + 7k_{1} c + \alpha_T) p_2 + k_{1} p_3 + \alpha_{T} p_{10},
\]

\[
\frac{dp_{10}(t)}{dt} = k_{1} p_{44} - 7k_{2} p_{45},
\]

where \( \alpha_B, k_{1}, etc. \) are the rate constants shown in Fig. 2. The value of \( c \) at any given time is related to the total concentration of S1, \( c_{S1} \), as

\[
c = c_{S1} - (c_{S1}^0 / 7) (\Lambda_3 + 2\Lambda_4 + 3\Lambda_5 + 4\Lambda_6 + 5\Lambda_7 + 6\Lambda_8 + 7\Lambda_9),
\]

where \( c_{S1}^0 \) is the total concentration of actin monomers in the system, and \( \Lambda_i \) represents the sum of all the \( p_i \) on the \( i \)th row in Fig. 3.

\[
\Lambda_3 = p_3 + p_{11} + p_{18},
\]

\[
\Lambda_4 = p_4 + p_{12} + p_{19} + p_{25},
\]

etc.

At \( t = 0 \), all units are at equilibrium and free of bound S1. Thus, it is easy to show that the only states in Fig. 3 with non-zero probabilities are states 1, 2, and 10:

\[
p_1(0) = 1/\Sigma,
\]

\[
p_2(0) = K_B/\Sigma,
\]

\[
p_{10}(0) = K_B K_T / \Sigma,
\]

where \( \Sigma = 1 + K_B (1 + K_T) \).

Eqs. 17–19 can be solved numerically with the initial probabilities given in Eqs. 23–25 at given \( c_{S1}^0 \) and \( c_{S1}^0 \).

After the \( p_i(t) \) are obtained, the time-dependent fractional saturation of the total actin sites \( \theta(t) \) can be evaluated as

\[
\theta(t) = \frac{1}{\Sigma} (\Lambda_3 + 2\Lambda_4 + 3\Lambda_5 + 4\Lambda_6 + 5\Lambda_7 + 6\Lambda_8 + 7\Lambda_9),
\]

where \( \Lambda_i \) is defined before in Eqs. 21, 22, etc. The fractional saturation of the pyrene fluorescence change \( R(t) \) can be evaluated as

\[
R(t) = \frac{1}{\Sigma} (\Lambda_3 + 2\Lambda_4 + 3\Lambda_5 + 4\Lambda_6 + 5\Lambda_7 + 6\Lambda_8 + 7\Lambda_9),
\]

where \( \Omega_i \) denotes the sum of all the \( p_i \) on the \( i \)th column in Fig. 3: \( \Omega_0 = p_{44}, \Omega_4 = p_{45} + p_{44}, etc. \)

MODEL CALCULATIONS

The main purpose of this paper is to examine whether the Hill model can reproduce the time course of S1 binding to actin as predicted by the Geeves model. Specifically, we wanted to examine whether both models account for the lag observed experimentally by McKillop and Geeves (1993) when mixing excess S1 to regulated actin in the absence of \( Ca^{2+} \). Thus, in this study, we first determined the set of kinetic parameters necessary for the Geeves model to reproduce the binding curves in Fig. 4 A of McKillop and Geeves (1993). Additional binding curves at different \( S1/actin \) ratios for the Geeves model were then calculated using these parameters and the question of whether the two models are kinetically equivalent was examined by fitting the Hill model to these binding curves.

Binding curves generated by the Geeves model

As discussed before, there are four kinetic reactions in the Geeves model: transitions between the blocked and the closed states and between the closed and the open state of the actin units and the two reactions of the two-step S1 binding. Two general rules must be obeyed when choosing the rate constants for model fitting. First, the ratio of the forward and the backward transition rate constants of any reaction must obey the equilibrium constants listed in Table 1 of McKillop and Geeves (1993). Therefore, there are only four independent rate constants to be found for the model. Second, the rate constants of the two-step S1 binding, \( k_{1}, k_{2}, k_{12}, k_{22}. \) and \( k_{3}, k_{4}, \) remain the same in the presence and in the absence of \( Ca^{2+} \) (McKillop and Geeves, 1993).

The kinetic parameters obtained necessary for the Geeves model to fit the curves in Fig. 4 A of McKillop and Geeves (1993) are listed in Table 1. A family of time courses of S1 binding to actin that differed in the concentration of S1 and actin are shown in Fig. 4 in dashed curves. At all concentrations of actin and S1, the Geeves model predicts that the observed rate of binding of S1 to actin is faster in the presence of \( Ca^{2+} \). In the cases where \( [S1] = [actin], \) the model predicts a lag in the binding in the absence of \( Ca^{2+} \) (Fig. 4 A and B). There is, however, little or no lag in the presence of \( Ca^{2+} \) or when \( [actin] > [S1] \) (Fig. 4 C).
Predictions of the Hill Model

Fitting the binding isotherm

To assign the kinetic rate constants of the Hill model, we first need to assign the equilibrium constants and the cooperativity parameters of the model: \( K_1, K_2, L', \) and \( Y. \) These parameters can be obtained by fitting the model to the experimental binding isotherms shown in Fig. 2, A and B of McKillop and Geeves (1993). Because the experimental binding data has been fit with the Geeves model (Table 1 of McKillop and Geeves, 1993), we derived the equilibrium constants by fitting the Hill model to Eq. 16 using the equilibrium constants listed in Table 1 of McKillop and Geeves (1993). The results are shown in Table 2. As one can see, the Hill model can fit the binding data of McKillop and Geeves with several sets of parameters. In general, the binding isotherm predicted by the Hill model is insensitive to \( K_1. \) In contrast, \( K_2, L', \) and \( Y \) are limited to a small range of values. It is also found that the values of \( Y \) in the presence and absence of \( Ca^{2+} \) are close to 1.

One must note that the values of \( L' \) and \( Y \) obtained here are quite different from those obtained by Hill et al. (1980) and by Williams and Greene (1983). In general, the \( L' \) evaluated here using the data of McKillop and Geeves is about 5–10 times larger than those obtained by others. In contrast, the value of \( Y \) is about 4–20 times smaller. These discrepancies may come from different methods used in measuring the binding isotherms.

Fitting the family of time courses of S1 binding to actin

As discussed before, there are six independent rate parameters for the Hill model. However, as shown in Table 2, the binding isotherm is insensitive to the value of \( K_1. \) Therefore, \( K_1 \) is also considered as an independent parameter in this case. Thus, there are a total of seven independent parameters to be determined in the modeling. One must note that \( Y_1 \) and \( Y_2 \) in Eqs. 13 and 14 are not independent parameters, because they can be evaluated from Eqs. 5 and 6 when the values of \( Y, \alpha_0, \) and \( \beta_0 \) are assigned.

After the values of the independent parameters are chosen, the kinetics of S1 binding is evaluated by Monte Carlo simulation. A set of parameters found to fit the calculated kinetic curves of the Geeves model at the three conditions in Fig. 4 is listed in Table 3. The simulated curves are shown as solid lines in Fig. 4 together with the results of the Geeves model simulation. As one can see from the figures, the kinetic curves calculated for the Hill model using the parameters in Table 3 are almost identical with those calculated for the Geeves model for the case where \([S1] \approx [actin]. \) Most importantly, the Hill model is also able to generate the lag. In the case where \([actin] > [S1], \) the Hill model predicts a slightly slower binding curve in the absence of Ca\(^{2+}\). However, this difference is too small to permit experimental discrimination between the two models.

DISCUSSION

The three- and two-state kinetic models generate similar time courses of S1 binding to regulated actin

As shown in Fig. 4, A–C, the two-state Hill model and the three-state Geeves model give very similar predictions for
the change in pyrene actin fluorescence upon the binding of S1. In the presence of Ca\(^{2+}\), the time course can be expressed by a one-exponential function at both high and low S1. Second, in the absence of Ca\(^{2+}\), the one-exponential kinetic behavior is generated only in the low S1 case. At high S1, the rate of fluorescence change is slow at the beginning of the reaction, forming a lag that precedes the rapid exponential phase. After the lag phase, the fluorescence increases exponentially. Third, when actin is in excess of S1, the rate of S1 binding is higher in the presence of Ca\(^{2+}\) than in the absence of Ca\(^{2+}\). The ratio of the apparent rate constants of the two curves is found to be \(\frac{4}{3}\), similar to that found experimentally. The Hill model differs slightly from the Geeves model only in the high actin case in the absence of Ca\(^{2+}\). Even under this condition, the difference is too small to allow distinction between the two models. The results shown here imply that the two models can be considered as kinetically equivalent in regards to the time course of S1 binding to regulated actin.

**Mechanism of generation of the lag in the Hill model**

The most important finding of this modeling work is the fact that the Hill model can generate the experimentally observed lag in the time course of S1 binding to regulated actin when an excess of S1 is mixed with actin in the absence of Ca\(^{2+}\). The generation of this lag by the Hill model depends on the kinetic parameters of the model in a complicated way. Here, we present a qualitative but general explanation of why the lag is present only in the case when Ca\(^{2+}\) is absent and S1 is in excess of actin and not in other experimental conditions.

In general, to generate a lag in the time course of pyrene fluorescence, S1 must remain unbound or bound in state 1 for some time before turning into state 2 at early times of the binding reaction. For example, if the reactions along the upper path (binding of S1 to actins in state 1) are slower than those in the lower path, then systems taking the binding route, path B in Fig. 5, will have a better chance of generating a lag than those taking path A. This is exactly the reason for the Hill model to generate the lag with the parameters in Table 3. At first, let us discuss the high S1 case. In Fig. 6, the net transition flux (forward minus backward) of each reaction step (S1 binding or transition between states 1 and 2) for a Tm–Tn–actin unit, as evaluated from the Monte Carlo simulation, is shown as a function of time. As shown in Fig. 6A, there are only four significant net fluxes with transitions starting from state 1 for the +Ca\(^{2+}\) case, \(J_{10} \rightarrow 11, J_{11} \rightarrow 21, J_{11} \rightarrow 12,\) and \(J_{12} \rightarrow 22\)

---

**TABLE 2 Equilibrium constants obtained by fitting the Geeves’ equilibrium binding data to the Hill model**

<table>
<thead>
<tr>
<th>Geeves Model</th>
<th>Hill model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(K_1)</td>
</tr>
<tr>
<td>+Ca(^{2+})</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>-Ca(^{2+})</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

The fitting was carried out using SigmaPlot. RMSD, root-mean-square-deviation. \(K_1, K_2,\) and \(K_{ij}\) are in units of \(\mu\text{M}^{-1}\). The others are unit-less.

**TABLE 3 Parameters of the Hill model used to fit the kinetic curves in Fig. 4 calculated for the Geeves model**

<table>
<thead>
<tr>
<th></th>
<th>+Ca(^{2+})</th>
<th>-Ca(^{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha_0)</td>
<td>200.0</td>
<td>200.0</td>
</tr>
<tr>
<td>(\beta_0)</td>
<td>300.0</td>
<td>300.0</td>
</tr>
<tr>
<td>(Y_1)</td>
<td>4.682</td>
<td>15.27</td>
</tr>
<tr>
<td>(Y_2)</td>
<td>0.208</td>
<td>0.0703</td>
</tr>
<tr>
<td>(k_1)</td>
<td>5.0</td>
<td>0.6</td>
</tr>
<tr>
<td>(k_2)</td>
<td>500.0</td>
<td>3.0</td>
</tr>
<tr>
<td>(k_3)</td>
<td>2.831</td>
<td>2.212</td>
</tr>
<tr>
<td>(k_4)</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>(\gamma)</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>(\delta)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

\(\alpha_0, \beta_0, k_1,\) and \(k_2\) are in units of \(s^{-1}\); \(k_1\) and \(k_2\) are in units of \(\mu\text{M}^{-1}s^{-1}\); The rest are dimensionless.
where \( i \to jm \) represents the transition from a unit in state \( i \) with \( n \) bound S1 to a state in \( j \) with \( m \) bound S1. The fact that \( J_{10 \to 11} \) and \( J_{11 \to 21} \) are almost identical and that they are much larger than \( J_{11 \to 12} \) and \( J_{12 \to 22} \) (specifically, \( J_{11 \to 21} > J_{11 \to 12} \)) implies that the main pathway of the S1 binding of the system is \( 10 \to 11 \to 21 \to 22 \to \cdots \) (path \( A \) in Fig. 5). In contrast, for the \(-Ca^{++}\) case \( J_{11 \to 12} \) is much larger than \( J_{11 \to 21} \), as shown in Fig. 6B. As a result, path \( B \) \((10 \to 11 \to 12 \to 22 \to \cdots)\) is the dominant pathway in this case. The flux curves in Fig. 6, \( C \) and \( D \) are consistent with the proposed pathways. For example, as one can see in Fig. 6 C, \( J_{21 \to 22} \) is the largest flux at the early times for the \(+Ca^{++}\) case, indicating that all the subsequent S1 binding in state 2 derives from the \( 11 \to 21 \) transition (path \( A \)).

**FIGURE 5** Possible binding pathways for the binding of S1 to a Tm–Tn–actin unit at the early times of the binding reaction. In path \( A \), a unit with one bound S1 changes from state 1 to state 2 before binding another S1. In path \( B \), the transition from state 1 to state 2 does not occur appreciably until the unit binds with two S1. Path \( B \) would produce a lag in the kinetic curve of pyrene-fluorescence and light scattering if the rate constant of binding S1 to actin in state 1 (those in the upper line) is smaller than that of binding to state 2.

**FIGURE 6** Net fluxes of some transition steps in the Hill model evaluated using the parameters in Table 3 for the high [S1] case: \( c_{S1}^0 = 2.5 \mu M \) and \( c_A^0 = 0.5 \mu M \). The first symbols, \( i \) and \( j \), in \( J_{in \to jm} \) represent the state of the unit, and the second symbols, \( n \) and \( m \), represent the number of bound S1. For example, \( 11 \to 21 \) represents the transition of a Tm–Tn–actin unit with one bound S1 from state 1 to state 2, etc. Net fluxes evaluated for the case with \( Ca^{++}\) present are shown in panels \( A \) and \( C \); those with \( Ca^{++}\) absent are shown in panel \( B \) and \( D \).
contrast, the fact that \( J_{22-23} \) is much larger than \( J_{11-22} \) at early times for the \(-\text{Ca}^{++}\) case means that the supply of \( J_{22-23} \) mainly comes from \( J_{12-22} \) (path B).

When the concentration of actin is much larger than S1 (the low S1 case), the number of S1 bound to a Tm–Tn unit in state 1 at early times of the reaction will be at most one, in both \(+\text{Ca}^{++}\) and \(-\text{Ca}^{++}\). That is, the probability of binding two or more S1 to a Tm–Tn–actin unit is very low in both cases. As a result, path A is the dominant pathway and no lag is expected.

It is important to point out that, as shown in Fig. 5, the S1 binding in the two-state Hill model involves many binding steps and many biochemical states, not just two as the word “two-state” implies. This is exactly why the model is able to generate the lag in the kinetic binding curve. There are even more states and more reaction steps in the Hill model if the binding of \( \text{Ca}^{++} \) is treated explicitly. In this case, modeling the lag in the kinetics of S1 binding is expected to be much easier.

**Lag also occurs in light scattering**

In this study, we were mainly concerned with the kinetic curves of pyrene fluorescence that measures the time course of S1-bound actin in the on state only, because this is the quantity measured by McKillop and Geeves (1993). As shown by Trybus and Taylor (1980), the lag was also found in the light scattering kinetic curve, which measures the total S1-bound actin in both on and off states as a function of time. The kinetics of light scattering can be easily calculated using the formalisms developed here. To see whether the light scattering could be used for model differentiation, we also carried out light-scattering calculations for the three cases in Fig. 4 using the same sets of parameters in Tables 1 and 3. We found that both models produced almost identical light-scattering kinetic curves (data not shown). A lag was also found for the case when excess S1 is mixed with actin in the absence of \( \text{Ca}^{++} \) for both models. However, the lag in light scattering is smaller (less sharper) than that in pyrene fluorescence (see the inset in Fig. 4 A). Why the Hill model is able to generate a smaller lag in light scattering in this excess-S1–no-Ca case can be explained also using the same arguments presented above for pyrene fluorescence. That is, processes taking path B in Fig. 5 are also expected to generate lags in light scattering, but not those taking path A. The reason for this is that the rate of binding of S1 to actin in state 1 (upper line in Fig. 5) is much slower than that in state 2 (lower line in Fig. 5), because \( k_1 < k_2 \) as shown in Table 3. Thus, although the binding of S1 to actin in state 1 also contributes to the light scattering signal, the time course of the signal is very different at early and late times, if the process takes path B: slow at first and fast later, thus forming a lag. The lag in light scattering is smaller than in pyrene fluorescence, because binding reactions of S1 to actin in state 1 in the upper line of Fig. 5 also contribute to the light scattering signal; in pyrene fluorescence, those reactions are completely silent.

**\( \text{Ca}^{++} \) effects on observed rate constants**

In fitting the Hill model to the kinetic curves generated by the Geeves model in Fig. 4, some of the parameters for the Hill model had to be \( \text{Ca}^{++} \) dependent. Specifically, the rate constants for attachment and detachment of S1 to actin in state 1 (the off state) have to be quite different in the presence and absence of \( \text{Ca}^{++} \), unlike those in the Geeves model (see Tables 1 and 3). There is experimental evidence for \( \text{Ca}^{++} \)-dependence of cross-bridge binding, at least in the presence of nucleotides. In the presence of ATPγS, the affinity of S1 to regulated actin is not significantly affected by \( \text{Ca}^{++} \) (Resetar and Chalovich, 1995). However, the rate of cross-bridge detachment, in single-muscle fiber preparations, is decreased in the presence of \( \text{Ca}^{++} \) (Kraft et al., 1992). The simulations done here were for the case where no nucleotide was present, so a direct comparison with experimental data is impossible. However, this \( \text{Ca}^{++} \)-dependence of the kinetics of binding may be a useful experimental approach to testing models of regulation. It will be particularly important in future studies to have accurate and independent determinations of the values of \( L' \) and \( Y \) under the conditions used for kinetic measurements.

**Potential value of the Hill model**

We have shown here that the Hill model is not at a disadvantage from other models in terms of describing the time course of binding. Recently, we have shown that the Hill model was able to simulate the activation kinetics of actin filaments in single fiber experiments (Brenner and Chalovich, 1999). Also, there are a sufficient number of physical states to accommodate the known structural changes of the regulated actin filament. The natural question is whether the Hill model has any advantages that would justify its continued study. The Hill model has been shown to predict the steady-state kinetics of ATP hydrolysis under a variety of conditions (Hill et al., 1981). The Geeves model describes the effects of \( \text{Ca}^{++} \) on the binding of myosin to actin but does not include changes in the rates of other transitions that might occur with changes in \( \text{Ca}^{++} \). Therefore, the ability of this model to predict steady-state kinetics of ATP hydrolysis cannot be rigorously examined. In the Geeves model, there is a significant change in the affinity of the S1–ATP state for binding to actin in the presence and absence of \( \text{Ca}^{++} \) (McKillop and Geeves, 1993). This occurs because of a \( >4 \)-fold change in the distribution of the closed state relative to the blocked state in the presence of \( \text{Ca}^{++} \). However, the actual difference in affinity of S1 for regulated actin during steady-state ATP hydrolysis between the high and low \( \text{Ca}^{++} \) conditions is two-fold or less (Chalovich et al.,
CONCLUSIONS

1. Mathematical procedures for the evaluation of kinetics of S1 binding to regulated actin filaments have been developed for both the two-state Hill model and the three-state Geeves model. These procedures are useful for the determination of the basic rate constants of the binding processes for both models when the kinetic curves of S1 binding are measured.

2. The two models yield qualitatively similar simulations of the time course of S1 binding to regulated actin measured using fluorescent pyrene-labeled actin. Specifically, the two-state Hill model can also generate the lag observed in the time course of S1 binding when excess S1 is mixed with actin in the absence of Ca$^{2+}$. As far as fitting the entire time-dependent kinetic curve of S1 binding to regulated actin filament is concerned, there is no difference between the Hill model and the Geeves model. Thus, the two-state model cannot be excluded based on equilibrium and pre-steady-state kinetics of S1 binding to regulated actin.

3. Using the same sets of kinetic parameters in Tables 1 and 3, we found that the Hill and the Geeves models also yielded similar light-scattering kinetics. Specifically, a lag was also found in the time course of light scattering when mixing excess S1 with actin in the absence of Ca$^{2+}$. Thus, light scattering kinetic curves also are not very useful in the differentiation of the two models.

4. The results obtained in this study indicate that the activation of regulated actin from the off to the on state in the absence of Ca$^{2+}$ may not require the existence of an intermediate state as required by the Geeves model. However, it is important to note that neither model has been rigorously tested for the ability to simulate multiple sets of experimental data.

The analyses presented in this paper are based on the equilibrium and kinetic data of McKillip and Geeves (1993) measured for nucleotide-free S1. The equilibrium constants derived for the Hill model using their data are greatly in discrepancy with those obtained by others (Hill et al., 1980; Williams and Greene, 1983). Whether the Hill and the Geeves models actually can or cannot be differentiated based on equilibrium and kinetic measurements of S1 binding may require matched and accurately measured equilibrium and kinetic binding curves of S1 with different bound nucleotides.

APPENDIX: EFFECT OF COOPERATIVITY ON TRANSITION RATE CONSTANTS BETWEEN STATES 1 AND 2

In this Appendix, Eqs. 13 and 14 will be derived based on the Transition Rate Theory of Eyring (Chang, 1981). According to this theory, transition rate constants, $\alpha_m$ and $\beta_m$, between states 1 and 2 in the absence of nearest-neighbor interactions (cooperativity) can be expressed as

$$\alpha_m = k \exp(- (E^* - E_1)/k_B T), \quad (A1)$$

$$\beta_m = k \exp(- (E^* - E_2)/k_B T), \quad (A2)$$

where $k$ is a constant, $E_1$ and $E_2$ are the free energies of an isolated unit in states 1 and 2, respectively, and $E^*$ is the free energy of the activated transition state of the reaction, as shown in Fig. 7. The factor $k_B T$ is the product of the Boltzmann constant and the temperature. In the presence of cooperativity, the energy profile of the reaction is altered. As shown in Fig. 7, let $\Delta E_1$, $\Delta E_2$, and $\Delta E^*$ represent the change of free energies of state 1, state 2, and the activated state, respectively. Then, the new rate constants $\tilde{\alpha}_m$ and $\tilde{\beta}_m$ can be obtained as

$$\tilde{\alpha}_m = k \exp(- (E^* + \Delta E^* - E_1 - \Delta E_1)/k_B T) = \alpha_m \exp(- (\Delta E^* - \Delta E_1)/k_B T), \quad (A3)$$

$$\tilde{\beta}_m = k \exp(- (E^* + \Delta E^* - E_2 - \Delta E_2)/k_B T) = \beta_m \exp(- (\Delta E^* - \Delta E_2)/k_B T), \quad (A4)$$

where Eqs. A1 and A2 have been used to obtain the second equality in both Eqs. A3 and A4. Now, let $N$ be the number of the two neighboring units.


