KINETIC MODEL FOR THE INTERACTION OF MYOSIN SUBFRAGMENT 1 WITH REGULATED ACTIN

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ABSTRACT A one-dimensional kinetic Ising model is developed to describe the binding of myosin subfragment 1 (SF-1) to regulated actin. The model allows for cooperative interactions between individual actin sites with bound SF-1 ligands rather than assuming that groups of actin monomer sites change their state in a cooperative fashion. With the triplet closure approximation, the model yields a set of 16 independent differential (master) equations which may be solved numerically to yield the extent of binding as a function of time. The predictions of the model are compared with experiments on the transient binding of SF-1 to regulated actin in the presence of Ca^{2+} and in the absence of Ca^{2+} with varying amounts of SF-1 prebound to the actin filament and on the equilibrium binding of SF-1 · ADP to regulated actin in the absence of Ca^{2+}. In all cases, the calculations fit the data to within the experimental errors. In the case of SF-1 · ADP, the results suggest that a repulsive interaction exists between adjacently bound SF-1 at the ends of two neighboring seven-site actin units.

INTRODUCTION

In a recent paper, Trybus and Taylor (1980) have presented experimental data on the transient-state kinetics of the binding of myosin subfragment 1 (SF-1) to regulated actin (the tropomyosin-troponin-actin complex, Tm-Tn-actin) in the presence and absence of Ca^{2+}. As in earlier equilibrium binding studies (Greene and Eisenberg, 1980), their observations demonstrate that a cooperative interaction arises when SF-1 binds to regulated actin. It is the purpose of this paper to analyze these data through the use of a one-dimensional kinetic Ising model, a model that allows for the investigation of dynamic cooperative phenomena. Though Hill et al. (1980) have formulated theoretical models for the equilibrium binding of SF-1 to regulated actin, there have been no previous attempts to model the transient-state binding.

In vertebrate striated muscle, the regulation of muscle contraction involves the interaction of four proteins: actin, myosin, troponin, and tropomyosin. The most widely accepted mechanism of troponin-tropomyosin action is the steric blocking model (Parry and Squire, 1973; Ebashi et al., 1969; Huxley, 1972; Haselgrove, 1972), which suggests that in the absence of calcium, the position of the tropomyosin molecule on the actin filament is such as to inhibit binding between actin and myosin. However, when Ca^{2+} binds to troponin, tropomyosin moves toward the central groove of the actin filament, making it possible for myosin to bind to actin. Because there is one tropomyosin molecule per seven actin monomer sites (McLachlan and Stewart, 1976), the action of the tropomyosin is thought to affect the entire seven site unit. To incorporate the steric blocking theory, Hill et al. (1980) have proposed a two-state binding model, where each tropomyosin-troponin unit (including seven actin sites for SF-1 binding) can be either in a "weak" or a "strong" binding state. In the weak binding state (binding constant K_1), SF-1 is sterically hindered from effective binding with actin, while in the strong binding state the interaction with actin is not blocked and SF-1 binds with a "strong" binding constant, K_2. Another aspect of the Hill model is that bound SF-1 molecules do not interact with one another. Rather, cooperative interaction occurs between nearest neighbor units of the regulated actin complex through the tropomyosins, and the strength of that interaction depends upon both the state of the unit and the number of Ca^{2+} ions (0, 1, or 2) bound to the troponin molecule in the unit. Thus, cooperativity results from the requirement that all seven sites in a unit change their state simultaneously and from the interaction between units.

Several experimental results have proven difficult to interpret with the above models. Those discrepancies have highlighted the fact that the nature or source of the cooperative response is still not well understood. Equilibrium binding studies by Chalovich et al. (1981) and Chalovich and Eisenberg (1981) have shown that at low $[Ca^{2+}]$ the binding constant of SF-1 · ATP or SF-1 · ADP · P_i to regulated actin is virtually the same as the binding constant at high $[Ca^{2+}]$ and the same as the binding constant of these molecules to unregulated actin. These results suggest that the troponin-tropomyosin complex does not inhibit the binding of SF-1 and consequently imply that the assumptions of the steric blocking model may require revision.

Trybus and Taylor (1980) find that some predictions of the Hill model do not agree with their kinetic data. They

suggest that a more realistic model would allow the bound tropomyosin molecule to exhibit flexibility, rather than rigid rodlike behavior, and a variety of states, rather than just the two allowed by the Hill model.

Recently, this model has been modified (Hill, 1981) to eliminate the dynamic equilibrium between empty (no SF-1 bound) Tm-Tn units in states 1 and 2. In the revised model, Tm-Tn is in state 1 if and only if no SF-1 is bound to the unit; Tm-Tn is in state 2 if and only if one or more SF-1's are bound to the unit. Transitions occur between states 1 and 2 only when the first SF-1 is bound to a unit or the last SF-1 is released from a unit. Thus, after the first SF-1 is bound to a unit with binding constant K_2/L, where the intrinsic equilibrium constant $L \geq 1$, subsequent SF-1's can bind more easily and have a binding constant $K_2 \geq K_2/L$. This "umbrella effect" generates the cooperativity observed in the equilibrium binding of SF-1 · ADP to regulated actin (Greene and Eisenberg, 1980; Hill et al., 1980). Hill (1981) concludes that there is a cooperative effect that favors the clustering of bound molecules, but that the molecules do not interact directly.

In the model we propose, it is the interactions between neighboring actin sites having bound SF-1's that contribute to the cooperativity of the system (rather than a simultaneous transition of a group of sites on the actin complex). Although our model is not based upon a specific physical picture of how the cooperativity arises, it is consistent with the experimental studies which suggest that the initial binding of SF-1 induces a conformational change (Loscalzo et al., 1975) or a cooperative activation (Trybus and Taylor, 1980) of the regulated actin filament which in turn affects how subsequent SF-1's bind.

MODEL

Analogous to the Hill (Hill et al., 1980) model, our treatment also takes the regulated actin unit to consist of seven binding sites, an assumption supported by structural evidence (McLachlan and Stewart, 1976). The features that characterize our model are (*a*) our assumption that the bound SF-1 ligands cluster in a cooperative manner and (*b*) our elimination of the two-state binding scheme. Though no direct evidence exists to support assumption *a*, it is consistent with the above view that the initial binding of SF-1 induces a change of the regulated actin filament that affects how subsequent SF-1's bind. We propose the following scheme for SF-1 ligand binding within a unit of seven sites. If a SF-1 ligand binds to a site with no occupied first nearest neighbors, it has a binding constant of k_f. If, however, the site to be occupied is contiguous to one occupied site in the same unit, the SF-1 ligand will bind with a binding constant of $k_f\omega_f$, where ω_f is the associative intraunit cooperativity factor. If the SF-1 ligand binds to a site with both contiguous neighbors in the same unit occupied, the binding constant is now $k_f\omega_f^2$. The dissociation rate constants for the corresponding situations are k_r, $k_r\omega_r$ and $k_r\omega_r^2$, respectively.

We introduce interunit cooperativity through parameters ω_f', ω_r' such that a site at the end of a unit has its forward (reverse) rate constant multiplied by the factor $\omega_f'(\omega_r')$ if its nearest neighbor in the adjacent unit is occupied. Values of $\omega_f' = \omega_r' = 1$ correspond to totally independent units, while values of $\omega_f'(\omega_r')$ greater than unity give rise to cooperative interunit effects, and values less than unity give rise to anti-cooperation interunit effects. If $\omega_f' = \omega_f$ and $\omega_r' = \omega_r$, the filament behaves as if there were no divisions between units. Fig. 1 illustrates some examples of the above prescription.

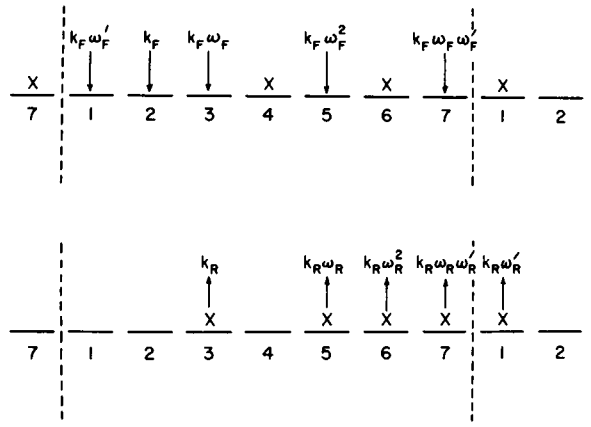

FIGURE 1 Associative and dissociative rate constants arising from the different types of processes. The numbers under the sites specify their location: site 7 is at the end of one unit, while site 1 is the first site of the adjoining unit. Each × represents a bound SF-1.

Using the scheme above, we are no longer constrained to view the unit as a rigid rod (seven sites changing state simultaneously) or constrained to the two-state binding model. Instead, our model can be viewed as displaying a variety of states; a state being defined by the number and distribution of SF-1 ligands bound to the unit.

Calcium ions may affect either the association or dissociation rates, k_f or k_r, the intraunit cooperativities, or the interunit cooperativities. Consequently, the effect of Ca^{2+} can be taken into account in our model by varying any one (or any number in combination) of these variables. The results obtained by Trybus and Taylor may help isolate which of these parameters is most affected by the calcium ions, or by any other relevant factor, such as ionic strength.

In common with the other one-dimensional models cited above, we neglect the interstrand actin interactions (each actin molecule in the thin filament has four nearest neighbors, two in the same strand and two in the neighbor strand). Though the effect of such interstrand interactions is not well documented, a model including these features remains an interesting topic for future research.

The model as summarized is analogous to an infinitely long Glauber model (Glauber, 1963) with an impurity at every seventh site. However, the fact that $k_f >> k_r$ (Trybus and Taylor, 1980) makes the situation comparable to a spin system in a magnetic field, where one cannot make the simplifying assumptions that the applied field is weak or that the system is near equilibrium. Without these conditions, the problem becomes analytically intractable. However, if certain other approximations are made, the resulting equations can be solved numerically.

Our approach is based upon the multiplet closure method (Schwarz, 1965; Silberberg and Simha, 1968), an approximation that neglects instantaneous correlations between lattice (actin) sites separated by more than some critical distance. When only first nearest neighbor cooperativity is taken into account, triplet closure is highly accurate in describing the kinetic behavior and is exact in describing the equilibrium characteristics (Schneider and Rawlings, 1973).

A triplet is defined as one of the possible states of a set of three adjacent sites on the (regulated actin) lattice. Each such set has eight (2^3) possible states (combinations of occupied and unoccupied sites), so that for a lattice consisting of seven-site units there would appear to be 56 (7.2^3) different triplets. However, symmetry considerations and sum rules (e.g., the sum of the eight triplet probabilities for each three-site group must be unity) reduce the number of independent triplet probabilities to 16. For an infinite undivided lattice ($\omega_f = \omega_f'$, $\omega_r = \omega_r'$) in which all sites are equivalent, the number of independent triplet probabilities is eight.

A differential equation (master equation) can be written for each of the independent triplet probabilities. This equation contains terms in involving quartet concentrations as well as triplets. An example is given in Eq. 1 for the triplet $(0\times\times)_4$ (see Fig. 1), where the numerical subscript denotes the location within the seven-site unit of the first site of the triplet. 0 and × denote vacant and occupied sites, respectively, and L is the free SF-1 concentration at time t:

$$\begin{aligned}\frac{d(0\times\times)_4}{dt} &= k_f\omega_f L(00\times)_4 + k_f\omega_f L(0\times00)_4 \\ &+ k_f\omega_f^2 L(0\times0\times)_4 \\ &+ k_r\omega_r(0\times\times\times)_3 + k_r\omega_r^2(\times\times\times\times)_3 \\ &- k_f\omega_f L(00\times\times)_3 \\ &- k_f\omega_f^2 L(\times0\times\times)_3 - k_r\omega_r(0\times\times)_4 \\ &- k_r\omega_r(0\times\times0)_4 \\ &- k_r\omega_r^2(0\times\times\times)_4. \end{aligned} \quad (1)$$

The triplet closure approximation consists of the elimination of all quartet terms in the differential equation by the substitution

$$(abcd)_n = \frac{(abc)_n(bcd)_{n+1}}{(bc)_{n+1}} \quad (2)$$

where a, b, c, d = × or 0 and

$$(bc)_{n+1} = (bc\times)_{n+1} + (bc0)_{n+1} = (\times bc)_n + (Obc)_n. \quad (3)$$

Eq. 2 is equivalent to the statement that there are no correlations extending beyond a range of three sites, while Eq. 3 is simply a statement that site n (or $n + 3$) must be either occupied (×) or unoccupied (0).

The computation is carried out by specifying the rate and cooperativity parameters k_f, k_r, ω_f, ω_r, ω_f', ω_r', the regulated actin concentration (in binding sites per liter), the initial SF-1 concentration L_0, and the initial triplet probabilities $(p_j)_0$. The sixteen coupled differential equations are then integrated numerically, and the extent of binding θ is calculated as

$$\theta(t) = \sum_{j=1}^{16} s_j n_j p_j(t)/28 \quad (4)$$

where s_j is the number of triplets equivalent in symmetry to the jth independent one, n_j is the number of sites (0, 1, 2, or 3) occupied in the jth triplet, and 28 is the normalization factor.

For binding to an initially bare lattice, all $(p_j)_0$ are zero, except those for triplets $(000)_n$, which are unity. In some experiments, the actin is first "preloaded" by allowing a prescribed amount of SF-1 to bind before initiating the stopped-flow experiment. These experiments are simulated in two stages: first, the appropriate quantity of SF-1 is allowed to bind to the initially vacant actin filament; second, the resulting p_j are used as the initial values for the binding to the preloaded actin.

RESULTS

To test the model described above, we attempted to simulate the results of three different experiments carried out by Trybus and Taylor (1980). In experiment 1, the binding kinetics of SF-1 to regulated actin in the absence of Ca^{2+} were followed without preloading and with preloaded filaments initially containing 1, 2, 3, 4, 5, and 6 bound SF-1 molecules per seven actin monomer sites. These data provide the most stringent test of our model. Experiment 2 was a kinetic study of the binding of SF-1 to regulated actin in the presence of Ca^{2+} with no preloading. In experiment 3, the equilibrium binding of SF-1 · ADP to regulated actin in the absence of calcium was determined as a function of the free ligand concentration.

For each experiment, the parameters were varied to obtain the optimal fit between simulation and experiment. In experiment 3, the equilibrium binding experiment, only the ratios $K = k_f/k_r$, $\omega = \omega_f/\omega_r$, and $\omega' = \omega_f'/\omega_r'$ affect the results, and the dissociative parameters k_r, ω_r and ω_r' were set to 1.0. The parameter values obtained are summarized in Table I. It should be noted that the large value of ω_f in experiment 1 and the small values of ω_r in experiments 1 and 2 are comparable to those observed in experiments on the association (Lohman and Kowalczykowski, 1981) and dissociation (Lohman, 1983) kinetics of T4 gene 32 helix-destabilizing protein with nucleic acids.

In Fig. 2 we compare our calculated results for the cases of 0, 1, 3, and 5 preloaded SF-1 per seven-site actin unit with Trybus and Taylor's data for experiment 1. (We thank Drs. K. Trybus and E. W. Taylor for making available the unpublished data for preloadings of 1, 3, 5, and 6). We see that the set of parameters given in Table I enables us to reproduce both the initial lag in the binding rate for low degrees of preloading and the disappearance of that lag as the preloading increases. In all seven cases our calculated results agree with the observed values at all times, to within the experimental error of ±0.04 θ. (Because the experimental data in Fig. 2 *C* and 2 *D* span a narrower range of values in θ, the expanded scale in these curves is different from the one used in Fig. 2 *A* and 2 *B*. Consequently, the error bars appear to be larger, but comparison with the scale on the θ axis reveals that these bars have the same value in all the figures.)

A similar comparison is shown in Fig. 3 for SF-1 binding in the presence of calcium. Again agreement is excellent. From a comparison of the parameter values in experiments 1 and 2, it appears that the absence of Ca^{2+} has two major effects. It introduces a strong interunit cooperativity that does not appear in the presence of calcium and it increases the associative intraunit cooperativity with a compensating decrease in the fundamental association rate. Calcium seems to have little or no effect on the dissociative parameters.

Experiment 3 probes the effect of attaching ADP to the SF-1 before binding. Again the calculated and experimental values agree well. The fit shown in Fig. 4 is slightly better than that obtained by Trybus and Taylor using the Hill model, though the parameters for the latter calcu-

TABLE I
OPTIMAL VALUES FOR PARAMETERS

Experiment	k_f	k_r	ω_f	ω_r	ω_f'	ω_r'
	$M^{-1}s^{-1}$	s^{-1}				
1	8,000	10	3,000	0.001	350	0.0095
2	160,000	10	75	0.001	1.0	1.0
3	1,500	1.0	405	1.0	0.1	1.0

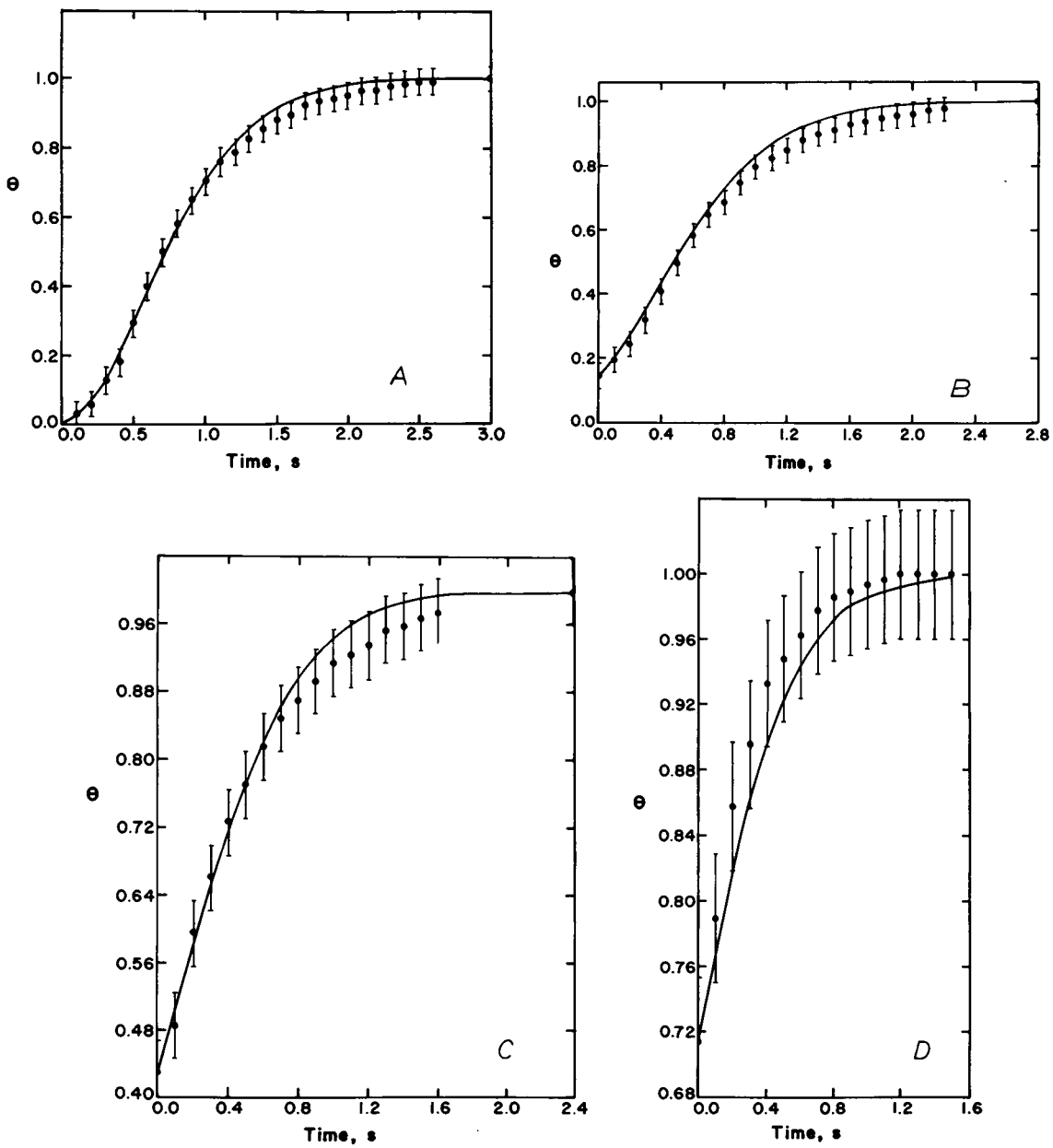

FIGURE 2 Transient binding kinetics of SF-1 to regulated actin in the absence of calcium. Experimental data are represented by the solid points (•), and error bars corresponding to ± 0.04 θ; solid curve represents calculated results with the parameters in Table I. *A*, SF-1 + regulated actin and no preloading; *B*, one SF-1 molecule per seven G-actin monomers is preloaded before mixing with an excess of SF-1; *C*, three SF-1 molecules preloaded per unit; and *D*, preloading of five SF-1's. Experimental conditions are given in Trybus and Taylor (1980). Due to the expanded scale in frames *C* and *D*, the error bars appear larger in these curves, however, their value remains the same throughout this figure.

lation were chosen so as to give agreement with the fluorescence curve as well as with the binding curve.

The model we have used contains six parameters, and it would appear at first glance that a search for an optimal set of parameters to fit a set of experimental data would be a difficult task at best. Fortunately, however, the different parameters exert their influence under quite different conditions. The most favorable case is k_f, which may be determined directly from appropriate kinetic data. At binding densities close to zero, essentially all molecules bind noncooperatively and dissociation is negligible. Thus, if one measures θ in the early moments of a nonpreloaded experiment, one has

$$k_f = \frac{1}{L_o} \lim_{t \to o} \frac{d\theta}{dt}. \quad (5)$$

The intraunit associative cooperativity ω_f controls the time of onset and the extent of the sharp increase in the binding rate that occurs once a sizeable number of SF-1

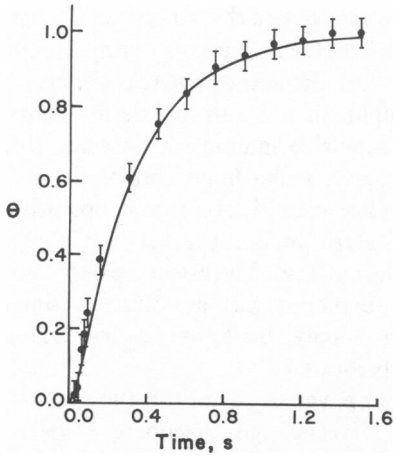

FIGURE 3 Transient binding kinetics of SF-1 to regulated actin in the presence of calcium. Experimental data are represented by the solid points (•), and error bars corresponding to ± 0.04 θ; curve is drawn through the theoretical results. The experimental conditions are the same as in Fig. 2.

molecules have become bound. When $\omega_f >> \omega_f'$, as is generally the case, the end sites of each unit tend to be occupied last, and ω_f' controls the rate at which these last "holes" are filled in ($\theta \rightarrow 1$).

In general, there is no simple relation between reported "equilibrium binding constants" and the parameters of this model. The equilibrium binding constant is a function of θ, given by $\bar{k}_f/\bar{k}_r$, where

$$\bar{k}_f = \sum_{i=1}^{16} s_i p_i k_{fi}/(1-\theta) \sum_{i=1}^{16} s_i p_i \quad (6)$$

$$\bar{k}_r = \sum_{i=1}^{16} s_i p_i k_{ri}/\theta \sum_{i=1}^{16} s_i p_i \quad (7)$$

where the s_i are the symmetry factors and the p_i are the equilibrium values of the triplet probabilities in Eq. 4. The quantities k_{fi} and k_{ri} are the rate constants appropriate to

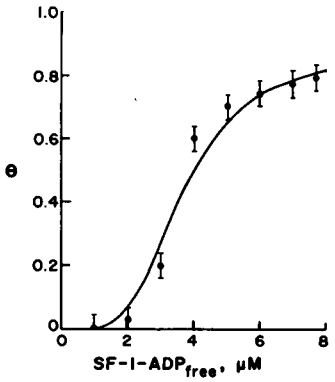

FIGURE 4 Equilibrium binding of SF-1 · ADP to regulated actin in the absence of Ca^{2+}. Experimental data are represented by the solid points (•), and error bars corresponding to ± 0.04 θ; curve is drawn through the calculated values. Experimental conditions are given in Trybus and Taylor (1980).

association or dissociation respectively, for the central site of the ith triplet. The p_i may be calculated given θ and the rate and cooperativity parameters from the formulas given by McGhee and von Hippel (1974).

Sample calculations show that $\bar{k}_f \approx k_f\omega_f$ for intermediate ($0.1 \lesssim \theta \lesssim 0.8$) occupancies and then rises sharply to the value $k_f\omega_f^2$ as $\theta \rightarrow 1$. The average dissociation rate $\bar{k}_r$ is nearly constant and equal to k_r until $\theta \gtrsim 0.5$, after which $\bar{k}_r$ drops toward its limiting value of $k_r\omega_r^2$.

The dissociative parameters k_r, ω_r and ω_r' have a significant influence on the results only when θ becomes large enough that

$$\bar{k}_r\theta \gtrsim \bar{k}_f L(1-\theta). \quad (8)$$

Because relation 8 is fulfilled only toward the very end of experiments 1 and 2, little information is obtained about these parameters, and they may be in error by an order of magnitude or more. A more accurate assessment of their values could be obtained in an experiment in which fully or nearly fully preloaded actin ($\theta_o \sim 1$) was mixed with a medium containing no SF-1.

The calculated kinetics are much more sensitive to the associative parameters, k_f, ω_f, and ω_f', particularly the first two. We estimate that k_f and ω_f can be varied individually by no more than 5% without significantly altering the fit between theory and experiment. In cases where initial rate data are not complete enough to fix k_f accurately, compensating changes in k_f and ω_f may be possible; for example, a 20% decrease in ω_f might roughly cancel the effects of a twofold increase in k_f.

DISCUSSION

To explain the increase in the SF-1 binding rate during the early part of the reaction, Trybus and Taylor (1981) invoke a two-state model for regulated actin. The model contains a strong-binding or open (active) state and a weak-binding or blocked (relaxed) state. They reason that because the rate is proportional to the concentration of available actin sites, there must be a net increase in the number of sites; therefore, SF-1 induces a cooperative transition from the relaxed to the active state of the filament.

In our model, the observed lag phase and subsequent increase in the rate of binding are explained in a different way. In our scheme, interactions between actin sites with bound SF-1's contribute to the cooperactivity of the system. After one SF-1 binds to an initially vacant filament, another SF-1 may bind to the neighboring site with greater facility. To explain the lag, we note that when the filament is preloaded with only 1 or 2 SF-1's, there is a relatively high probability that subsequent SF-1's will not bind adjacent to already occupied sites. However, as the preloading increases to and above three (which corresponds to approximately one SF-1 molecule per two actin monomer sites), the probability of binding adjacent to an already

bound site increases and thus binding to the lattice is facilitated. Consequently, the lag disappears, not because the number of available sites increases, but because a higher fraction of unoccupied sites have their binding constant enhanced by interaction with neighboring occupied sites.

Our model is also consistent with the equilibrium studies by Greene and Eisenberg (1980), who found that binding of SF-1 · ADP to regulated actin in the presence of absence of Ca^{2+} is cooperative. Whether or not calcium was present, the binding strength of SF-1 · ADP to regulated actin was greater at high levels of occupancy of regulated actin by SF-1 · ADP than at low levels of occupancy. In other words, when a filament is already occupied to a high degree, further SF-1 · ADP binds to a site with a stronger binding constant than when the filament is sparsely occupied. As the regulated actin becomes highly occupied, the probability of finding a vacant site with two contiguous occupied neighbors increases relative to the probability of finding a vacant site with one or zero contiguous occupied neighbors. In our model, the binding constant for the first case is $k_f\omega_f^2$ (considering, for the moment, only intraunit cooperativity, the more frequently occurring interaction), and $k_f\omega_f$, and k_f, respectively, for the other cases. Because a good fit with experimental data requires a value of $\omega_f >> 1$, we have $k_f\omega_f^2 >> k_f\omega_f >> k_f$, where SF-1 · ADP binding to a sparsely occupied lattice will typically have a binding constant between $k_f\omega_f$ and k_f.

Our result that the binding of SF-1 to regulated actin in the presence of Ca^{2+} is less cooperative than in the absence of Ca^{2+} is in agreement with the equilibrium observations of Greene and Eisenberg (1980) on the binding of SF-1 · ADP to regulated actin. Our calculated value of k_f for SF-1 binding to regulated actin in the presence of Ca^{2+} is 2×10^1 greater than that in the absence of Ca^{2+} while k_r is the same in both cases. This result is consistent with the observation of Trybus and Taylor (1980) that the difference in initial slopes in the presence and absence of Ca^{2+} is approximately one order of magnitude.

One surprising result emerges from our fit of the equilibrium binding curve of SF-1 · ADP to regulated actin (experiment 3, Fig. 4). For the curve to reach the value of $\theta \approx 0.8$ at the observed free ligand concentrations, the value of ω_f had to be set equal to 0.1. This value indicates an anticooperative or repulsive interaction between the adjacent SF-1 molecules bound in positions 7 and 1, i.e., at the point of overlap between two adjacent Tm-Tn units. However, it is just at this point in the filament where a head-to-tail overlap of about nine residues occurs in the tropomyosin molecules (Phillips et al., 1979). Further, Phillips et al. (1979) suggest that the tropomyosin does not maintain the α-helical conformation at the ends, but rather the chains are joined by intermeshing to form a small globular domain. Our results indicate that two SF-1 · ADP, binding to adjacent head and tail positions, are sensitive to this structural change. We might infer that this "defect," prevents optimal binding between SF-1 · ADP and the actin sites at positions 7 and 1. This situation might then result in steric hindrance and a consequent repulsive interaction between these adjacent species. Recently, Nagashima and Asakura (1982) have reported another example of an anticooperative interaction arising from steric hinderance between two SF-1 species bound to adjacent sites. The effect we describe above is not propagated further down the filament since the head-to-tail overlap is only about nine residues long and thus the effect is fairly localized.

Though we have not tried to fit the specific data points obtained by Greene and Eisenberg (1980) (performed under different conditions than those used by Trybus and Taylor), our results suggest that decreasing our value of k_f and increasing our value of ω_f would reproduce the salient features of their equilibrium binding curve for SF-1 · ADP to regulated actin in the absence of Ca^{2+}. These features are (in contrast with the points in Fig. 4) (*a*) a longer, flatter lag phase at the beginning of the curve, indicating a lower value of k_f, and (*b*) a sharper rise in the extent of binding, indicating a higher degree of cooperativity.

Finally, we point out that the method utilized here yields the triplet probabilities at all times during the course of the reaction, from which one could calculate cluster distributions of open or occupied sites or similar quantities. In several of the cases studied here, the triplet probabilities for a given θ during a transient experiment differ from the corresponding quantities at the same θ at equilibrium. This observation suggests the possibility of hysteresis effects, and will be investigated further.

In summary, our results suggest that a one-dimensional Ising model with first nearest neighbor cooperativity, provides a promising model for describing the kinetics of SF-1-regulated actin interactions. We would emphasize both that kinetic data like those analyzed here provide a far more stringent test of a model than do equilibrium data, and that there is a need for more extensive data of this type, particularly for conditions in which the dissociative processes play a significant role.

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REFERENCES

Chalovich, J. M., P. B. Chock, and E. Eisenberg. 1981. Inhibition of actomyosin ATPase activity without inhibition of myosin binding to actin. *J. Biol. Chem.* 256:575–578.

Chalovich, J. M., and E. Eisenberg. 1981. Further studies on the binding of myosin subfragment-1 to regulated F-actin in the presence of ATP. *Biophys. J.* 33(2,Pt.2):232 *a*.(Abstr.)

Ebashi, S., M. Endo, and I. Ohtsuki. 1969. Control of muscle contraction. *Q. Rev. Biophys.* 2:351–384.

Glauber, R. J. 1963. Time-dependent statistics of the Ising model. *J. Math. Phys. (Cambridge, MA)*. 4:294–309.

Greene, L. E., and E. Eisenberg. 1980. Cooperative binding of myosin subfragment-1 to the actin-troponin-tropomyosin complex. *Proc. Natl. Acad. Sci. USA*. 77:2616–2620.

Haselgrove, J. C. 1972. X-ray evidence for a conformational change in the actin-containing filaments of vertebrate striated muscle. *Cold Spring Harbor Symp. Quant. Biol.* 37:341–352.

Hill, T. L. 1981. Binding under a molecular "umbrella" as a cooperative statistical mechanical system: tropomyosin-actin-myosin as an example. *Biophys. Chem.* 14:31–44.

Hill, T. L., E. Eisenberg, and L. E. Greene. 1980. Theoretical model for the cooperative equilibrium binding of myosin subfragment 1 to the actin-troponin-tropomyosin complex. *Proc. Natl. Acad. Sci. USA*. 77:3186–3190.

Huxley, H. E. 1972. Structure changes in the actin- and myosin-containing filaments during contraction. *Cold Spring Harbor Symp. Quant. Biol.* 37:361–376.

Lohman, T. M. 1983. A model for the irreversible dissociation kinetics of peratively bound protein-nucleic acid complexes. *Biopolymers*. 22:1697–1713.

Lohman, T. M., and S. C. Kowalczykowski. 1981. The association kinetics of the bacteriophage T4 gene 32 (helix destabilizing) protein with single stranded nucleic acids. Evidence for protein translocation. *J. Mol. Biol.* 152:67–102.

Loscalzo, J., G. H. Reed, and A. Weber. 1975. Conformational change and cooperatively in actin filaments free of tropomyosin. *Proc. Natl. Acad. Sci. USA*. 72:3412–3415.

McGhee, J. D., and P. H. von Hippel. 1974. Theoretical aspects of DNA-protein interactions: cooperative and non-cooperative binding of large ligands to a one-dimensional homogeneous lattice. *J. Mol. Biol.* 86:469–489.

McLachlan, A. D., and M. Stewart. 1976. The 14-fold periodicity in α-tropomyosin and the interaction with actin. *J. Mol. Biol.* 103:271–298.

Nagashima, H., and S. Asakura. 1982. Studies on co-operative properties of tropomyosin-actin and tropomyosin-troponin-actin complexes by the use of *N*-ethylmalemide-treated and untreated species of myosin subfragment 1. *J. Mol. Biol.* 155:409–428.

Parry, D. A. D., and J. M. Squire. 1973. Structural role of tropomyosin in muscle regulation: analysis of the x-ray diffraction patterns from relaxed and contracting muscles. *J. Mol. Biol.* 75:33–55.

Phillips, G. N., Jr., E. E. Lattman, P. Cummins, K. Y. Lee, and C. Cohen. 1979. Crystal structure and molecular interactions of tropomyosin. *Nature (Lond.)*. 278:413–417.

Schneider, F. W., and P. K. Rawlings. 1973. Kinetics of the one dimensional lattice and triplet closure. *Ber. Bunsen-Ges. Phys. Chem.* 77:237–243.

Schwarz, G. 1965. On the kinetics of the helix-coil transition of polypeptides in solution. *J. Mol. Biol.* 11:64–77.

Silberberg, A., and R. Simhą. 1968. Kinetics of reversible reactions on linear lattices with neighbor effects. *Biopolymers*. 6:179–190.

Trybus, K. M., and E. W. Taylor. 1980. Kinetic studies of the cooperative binding of subfragment 1 to regulated actin. *Proc. Natl. Acad. Sci. USA*. 77:7209–7213.