KINETICS OF ACTIN–MYOSIN BINDING

I. An Exactly Soluble One-variable Model

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ABSTRACT To treat the kinetics of actin–myosin binding as simply as possible, a one-variable model is developed and the notion of effectivity factors is introduced. An effectivity factor is a ratio of the reaction rate in the presence of cooperativity to that in the noncooperative case and is calculated by averaging cooperativity factors over all sites belonging to one seven-site actin unit. The technique is applicable to a variety of models involving cooperative association and dissociation processes. This averaging assumes the equivalence of all regulated actin units. The model may be solved exactly for arbitrary degrees of "preloading" of subfragment 1 (S1) on the regulated actin.

INTRODUCTION

The binding of myosin to actin is a crucial step in the contraction of muscle. While the equilibrium binding has been thoroughly investigated both experimentally (Greene and Eisenberg, 1980) and theoretically (Hill et al., 1980), studies of the transient-state binding have been much more limited.

Balazs and Epstein (1983) proposed a one-dimensional kinetic quasi-Ising model to treat the kinetics of myosin subfragment 1 (S1) binding to regulated actin (the troponin–tropomyosin–actin complex). The results obtained were in good agreement with the stopped flow experiments of Trybus and Taylor (1980), the most thorough investigation of the kinetics of actin–myosin binding to date. Simulations were carried out of binding in the presence and absence of Ca^{2+} and with and without prescribed amounts of S1 prebound to the regulated actin.

While the Balazs-Epstein model appears to be able to explain a variety of data, it is computationally unwieldy. It requires the numerical integration of a set of 16 coupled differential equations that, for some sets of parameters, may become numerically stiff (Gear, 1971), requiring inordinate amounts of computer time to yield satisfactory accuracy. For binding the two-headed subfragment heavy meromyosin (HMM), the number of equations rises to 36, and the computational effort becomes prohibitive for all but numerical analysts. We present here a simplified model that incorporates the major assumptions of the earlier scheme, most notably allowing cooperativity between individual actin sites within an actin unit rather than requiring each unit to change its state as a whole (Hill et al., 1980). The present model uses an averaging technique to limit sharply the number of variables. In the version presented here, S1 binding is described by a single differential equation that may be solved analytically. It is our hope that the simplicity of the model will make it sufficiently attractive so that it will be of practical use to experimentalists in analyzing their data and in devising their experiments.

A SIMPLE KINETIC MODEL

We assume that actin consists of regulated units, each one containing seven sites (McLachlan and Stewart, 1976), to which S1 fragments bind in a cooperative manner (Balazs and Epstein, 1983). There are A_o actin sites and M_o myosin sites (one for each S1 fragment). All actin regulated units are assumed to be equivalent, i.e., the effects of the finite length of the actin chains or, equivalently, the influence of the molecular weight distribution of actin are assumed to be negligible. The total number of regulated units is R, where

$$R = A_o/7. \tag{1}$$

A_r actin sites are bound to myosin and the remaining A_f sites are free

$$A_r + A_f = A_o. \tag{2}$$

The fraction of reacted actin sites is the main variable of the model

$$\theta = A_r/A_o. \tag{3}$$

We also introduce as a parameter the actin-to-myosin stoichiometric ratio

$$\gamma = \frac{A_o}{M_o} \tag{4}$$

At a given time there are M_r S1 fragments bound to

actin whereas M_f are still free. Of course

$$M_r = A_r, \tag{5}$$

$$M_f = M_o - M_r. \tag{6}$$

Using Eqs. 3 and 4 one obtains from Eqs. 5 and 6

$$M_f = M_o(1 - \gamma\theta) = A_o[(1/\gamma) - \theta]. \tag{7}$$

The actual rate of myosin binding is a sum of individual rates for all free actin sites in all the regulated units

$$V_f = \sum_{i=1}^{A_f} v_{fi}. \tag{8}$$

The individual rates, v_{fi}, depend both on the position of the ith free binding site in the regulated unit it belongs to and on the states (free or bound) of the two nearest-neighbor active sites (Balazs and Epstein, 1983)

$$v_{fi} = k_f M_f Q^f(i - 1)Q^f(i + 1), \tag{9}$$

where $k_f M_f$ is the rate of binding of actin sites reacting independently of one another and the Q^f's are binding coupling factors.

Because of the equivalence of all actin regulated units, we first average individual binding rates over one unit.

$$v_f = k_f M_f e_f, \tag{10}$$

where

$$e_f = \frac{1}{7}\sum_{l=1}^{7}[Q^f_{(l-1)}Q^f_{(l+1)}] \tag{11}$$

will be called the binding effectivity factor and

$$Q^f_k = (1 - p_k) \tag{12a}$$

if the kth site is still free, whereas

$$Q^f_k = \omega^f_k p_k \tag{12b}$$

if the kth site is bound to an S1. For $l = 1$ and $l = 7$ the actin site is affected by a neighbor belonging to another regulated unit and we treat the index k modulo 7.

The term p_k denotes the probability that the kth site in a regulated unit is bound. To obtain our simple one variable model, we assume

$$p_k = \theta \tag{13}$$

for all k. The cooperativity factor ω^f_k (Balazs and Epstein, 1983) is defined to take into account the inequivalence of interactions between neighboring sites in the same unit and sites in different, but adjacent units:

$$\omega^f_k = \omega_f \text{ for } k = 2, 3, 4, 5, 6 \text{ (internal sites)}, \tag{14a}$$

$$\omega^f_k = \omega'_f \text{ for } k = 1, 7 \text{ (external sites)}. \tag{14b}$$

Taking into account Eqs. (12–14) one obtains

$$e_f = \frac{1}{7} \cdot [7 \cdot (1 - \theta)^2 + 5 \cdot \{2\} \cdot \omega_f\theta(1 - \theta) + 2 \cdot \omega_f\theta(1 - \theta) + 2 \cdot \omega'_f\theta(1 - \theta) + 5 \cdot \omega_f^2\theta^2 + 2 \cdot \omega_f\omega'_f\theta^2]. \tag{15}$$

The terms on the right hand side of Eq. 15 represent the following, from left to right: sites with both neighbors free, internal sites with one neighbor free and one bound (note the combinatorial factor of $\{2\}$), external sites with the neighbor in the same unit bound and the neighbor in the adjacent unit free, external sites with the neighbor in the same unit free and the neighbor in the adjacent unit bound, internal sites with both neighbors bound, and, finally, external sites with both neighbors bound.

It is obvious from the definition (Eq. 11) that the effectivity factor is a ratio of the reaction rate in the presence of cooperativity to that in the noncooperative case. That is, e_f is nothing but the cooperativity factors averaged over all sites belonging to one regulated actin unit. For a noncooperative system ($\omega_f = \omega'_f = 1$), the effectivity factor is, of course, unity.

Using Eq. 10 one may write the total rate of myosin binding, Eq. 8, in the form

$$V_f = A_f v_f. \tag{16}$$

Combining Eqs. 2, 3, 7, 10, and 16, we obtain

$$V_f = A_o(1 - \theta) \cdot k_f \cdot M_o(1 - \gamma\theta) \cdot e_f. \tag{17}$$

The total rate of dissociation of actomyosin linkages may be found in an exactly analogous manner, with dissociative cooperativities, coupling, and effectivity factors (with r subscripts) replacing their associative counterparts in Eqs. 8, 9, 11, and 16. The dissociation effectivity factor, e_r, is given by

$$e_r = \frac{1}{7} \cdot [7 \cdot (1 - \theta)^2 + 5 \cdot \{2\} \cdot \omega_r\theta(1 - \theta) + 2 \cdot \omega_r\theta(1 - \theta) + 2 \cdot \omega'_r\theta(1 - \theta) + 5 \cdot \omega_r^2\theta^2 + 2 \cdot \omega_r\omega'_r\theta^2]. \tag{18}$$

Taking into account Eq. 3, one obtains

$$V_r = A_o\theta \cdot k_r \cdot e_r. \tag{19}$$

The total rate of change of the number of acto–myosin linkages is given by the difference of V_f and V_r,

$$\frac{dA_r}{dt} = V_f - V_r \tag{20}$$

Dividing Eq. 20 by A_o and using Eqs. 3, 17, and 19, we

have

$$d\theta/dt = M_o(1 - \gamma\theta)(1 - \theta)k_f e_f - \theta k_r e_r, \quad (21)$$

where e_f and e_r are given by Eqs. 15 and 18, respectively.

The major advantage of the model is evident in Eq. 21. The right-hand side is a quartic polynomial in θ and thus, as shown in the next section, may be integrated analytically. The expressions obtained, though complex, may easily be evaluated on a programmable pocket calculator. It thus becomes feasible the test a wide range of rate and cooperativity parameters. More elaborate versions of the model, in which Eq. 13 is replaced by more accurate assumptions about the site probabilities, will be considered in Parts II and III of this series.

ANALYTIC SOLUTION

On inserting Eqs. 15 and 18 in Eq. 21, we find that the right-hand side of Eq. 21 may be written in the form

$$d\theta/dt = x(\theta) = Q\theta^4 + U\theta^3 + V\theta^2 + W\theta + Z, \quad (22)$$

where

$$Q = \gamma(k_f M_o - A_f + B_f), \quad (23a)$$

$$U = A_f + A_r - (B_f + B_r) - (k_r + k_f M_o) + \gamma(-3k_r M_o + 2A_f - B_f), \quad (23b)$$

$$V = -(2A_f + A_r) + B_f + 3k_f M_o + 2k_r + \gamma(3k_f M_o - A_f), \quad (23c)$$

$$W = A_f - (3k_f M_o + k_r) - \gamma k_f M_o, \quad (23d)$$

$$Z = k_f M_o, \quad (23e)$$

and

$$A_i = 2k_i(\tfrac{6}{7}\hbar\omega_i + \tfrac{1}{7}\hbar\omega_i'), \quad (24a)$$

$$B_i = k_i(\tfrac{5}{7}\hbar\omega_i^2 + \tfrac{2}{7}\hbar\omega_i\omega_i'), \quad i = r, f \quad (24b)$$

$$k_f = k_f M_o, \quad (24c)$$

$$k_r = k_r. \quad (24d)$$

Eq. 22 must have one real root, r_1, lying between 0 and 1 and at least one additional real root, r_2. We may thus factor $x(\theta)$ in the form

$$x(\theta) = (\theta - r_1)(\theta - r_2)(a\theta^2 + b\theta + c), \quad (25)$$

where

$$a = Q, \quad (26a)$$

$$b = U + Q(r_1 + r_2), \quad (26b)$$

$$c = \frac{Z}{r_1 r_2}. \quad (26c)$$

Let θ_0 be the initial value of θ at t = 0. Then Eq. 22 may be integrated exactly via a partial fraction expansion of $x(\theta)$ in Eq. 25

$$dt = \frac{d\theta}{x(\theta)} = \left(\frac{\alpha}{\theta - r_1} + \frac{\beta}{\theta - r_2} + \frac{\theta + \delta\theta}{a\theta^2 + b\theta + c}\right)d\theta, \quad (27)$$

with

$$\alpha = \frac{1}{(r_1 - r_2)(ar_1^2 + br_1 + c)}, \quad (28a)$$

$$\beta = \frac{1}{(r_2 - r_1)(ar_2^2 + br_2 + c)}, \quad (28b)$$

$$\gamma = \frac{b^2 - ac + ab(r_1 + r_2) + a^2 r_1 r_2}{(ar_1^2 + br_1 + c)(ar_2^2 + br_2 + c)}, \quad (28c)$$

$$\delta = \frac{a^2(r_1 + r_2) + ab}{(ar_1^2 + br_1 + c)(ar_2^2 + br_2 + c)}. \quad (28d)$$

The result of integrating Eq. 27 is

$$t = \alpha \ln\left(\frac{\theta - r_1}{\theta_0 - r_1}\right) + \beta \ln\left(\frac{\theta - r_2}{\theta_0 - r_2}\right) + \left(\frac{\delta}{2a}\right)\ln\left(\frac{a\theta^2 + b\theta + c}{a\theta_0^2 + b\theta_0 + c}\right) + \left(\gamma - \frac{b\delta}{2a}\right)f(\theta), \quad (29)$$

where

$$f(\theta) = \begin{cases} \dfrac{2}{(-\Delta)^{1/2}}\arctan\left(\dfrac{2a\theta + b}{(-\Delta)^{1/2}}\right) - \arctan\left(\dfrac{2a\theta_0 + b}{(-\Delta)^{1/2}}\right) & \text{if } \Delta < 0, \\ \dfrac{4a(\theta - \theta_0)}{(2a\theta + b)(2a\theta_0 + b)} & \text{if } \Delta = 0, \\ \dfrac{1}{\Delta^{1/2}}\ln\left[\dfrac{(2a\theta + b - \Delta^{1/2})}{(2a\theta + b + \Delta^{1/2})} \cdot \dfrac{(2a\theta_0 + b + \Delta^{1/2})}{(2a\theta_0 + b - \Delta^{1/2})}\right] & \text{if } \Delta > 0 \end{cases} \quad (30)$$

with $\Delta = b^2 - 4ac$.

The expression in Eq. 29 is certainly cumbersome, but it is easily evaluated even on a programmable pocket calculator, first by finding the roots r_1 and r_2 (e.g., by Newton's method), and then by evaluating successively Eqs. 26, 28, and 29. The result of the calculation for a typical set of parameters is shown in Fig. 1.

In the limiting case of a large excess of myosin ($\gamma \ll 1$), the quartic term Q in Eq. 22 may be neglected, and we have a cubic polynomial, which considerably simplifies the calculation.

DISCUSSION

The present model may be thought of as derived from that of Balazs and Epstein (1983) by a singlet closure approximation (Schwarz, 1965). That is, instead of considering triplet probabilities, one now considers only the probability

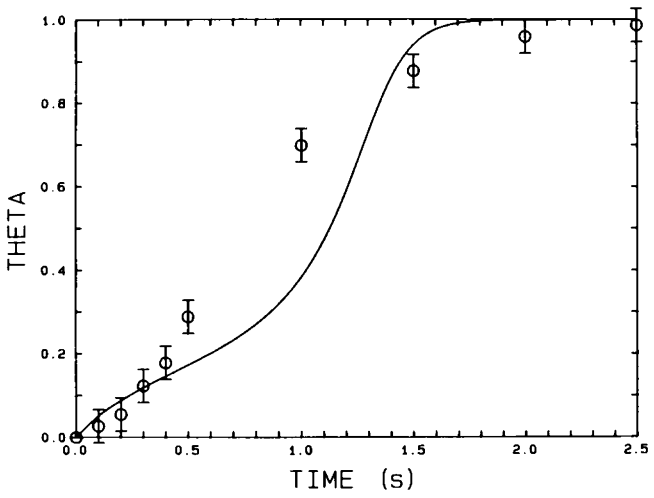

FIGURE 1 Extent of binding of myosin subfragment 1 to regulated actin in the absence of Ca^{2+}. Experimental conditions: 20°C, 10 mM Tris MES (pH 7), 0.2 M KCl, 5 mM $MgCl_2$, 2 mM EGTA, 10^{-6} M actin, 4×10^{-6} M S1. Points: experimental data (Trybus and Taylor, 1980). *Solid line*, calculated from Eq. 29 with $k_f = 1.6 \times 10^5\ M^{-1}\ s^{-1}$, $k_r = 10\ s^{-1}$, $\omega_f = 6$, $\omega_r = 0.001$, $\omega_f' = \omega_r' = 1$.

that a site is bound or free, regardless of the states of its neighbors. The simplified model also neglects differences in site probabilities between different sites within a regulated unit. These approximations appear rather drastic. However, close examination of the individual site probabilities obtained in the more complex model shows that the assumption of equal site probabilities made in Eq. 13 is reasonably accurate so long as ω_f does not differ from ω_f' (and ω_r does not differ from ω_r') by more than about two orders of magnitude.

The advantages of a mode that affords an analytical solution without significant loss of accuracy are considerable. Further papers in this series will show how the model may be elaborated to give a more accurate description of S1 binding as well as to treat more complex situations such as HMM binding.

We thank Anna Balazs for providing detailed computational results on the earlier model and Johannes Reiter for assistance in preparing the figure.

This work was supported by National Institutes of Health grant AM-31600 and by National Science Foundation grant PCM-8302350.

Received for publication 7 March 1986 and in final form 23 June 1986.

REFERENCES

Balazs, A. C., and I. R. Epstein. 1983. Kinetic model for the interaction of myosin subfragment 1 with regulated actin. *Biophys. J.* 44:145–151.

Gear, C. W. 1971. Numerical Initial Value Problems in Ordinary Differential Equations. Prentice-Hall, Inc., Englewood Cliffs, NJ. Chapt. 11. 209–230.

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

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.

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.

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

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