Biophysical Journal, Volume XX

Supporting Material for "Cooperative [Ca^{2+}]-dependent regulation of the rate of myosin binding to filamentary actin; solution data and the chain model."

Appendix A: Steric slowing of myosin binding with excess myosin.

Michael Geeves,[‡] Hugh Griffiths,[‡] Srboljub Mijailovich[*] and David Smith.[#]

[‡] Dept. of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, U.K..
[*] Dept. of Environmental Health, Harvard School of Public Health, Boston MA 02115.
[#] Department of Zoology, La Trobe University, Melbourne, Victoria 3086, Australia.

There are numerous reports of myosin-actin binding transients being well described by a two-exponential fit, with fast and slow components. When using excess S1, both components are linear in the myosin concentration. If excess actin is mixed with S1, the rate constant of the fast component is similar to that for excess S1, but the slow component has a greatly reduced amplitude and can be absent. This is true of pure actin filaments and actin.TmTn filaments. The presence of the slow phase with excess myosin is probably due to progressive steric blocking of actin sites by bound myosins, or possibly to the tendency of actin and myosin filaments at high concentrations to gel or aggregate, which would slow the diffusion of free myosins. The degree of gelling can vary from preparation to preparation, making precise quantitative analysis difficult. Such effects are smaller, but still present, when monitoring the fluorescence of a pyrene label attached to actin rather than light scattering.

For the excess actin.Tm.Tn data presented here, the slow component is small and does not change with calcium concentration so it can be easily corrected in the data sets. For excess S1 binding to actin.Tm.Tn, the transients show a pronounced lag which prevents a simple multi-exponential fit, so this condition was also investigated with unregulated actin. Rapid mixing excess S1 with pure actin filaments gave transients fitted by two exponentials of similar amplitude and rate constants that differed by a factor of 3-4. If the actin was preloaded with S1 so that 0-80% of the sites were already occupied before mixing, then the same two transients were observed but the amplitude of the fast phase was reduced while the slow phase remained constant, until at ~80% saturation the fast phase was absent, leaving just a slow phase. We therefore tried the same approach with actin.Tm.Tn and excess S1. Trybus and Taylor (A1) had already reported that the lag phase in the excess S1 transient could be titrated away by preincubating the actin with small amounts of S1. At 80% saturation we observed a single exponential phase for the transient that was both independent of calcium and almost identical to the phase seen for pure actin filaments. Thus the transients obtained at 80% pre-loading could have been used to estimate the slow component and subtract it from the data; however, pre-loading would modify the effects of chain-induced cooperativity which we sought to model.

Because the amplitude of the slow phase is significant only with excess myosin, we conclude that the slow phase arises from steric slowing rather than the onset of gelation. While a two-exponential fit is adequate for myosin binding to unregulated actin, similar fits to the post-lag phase of binding to regulated actin required calcium-dependent rate constants, which is not consistent with any simple picture of steric slowing. Hence steric slowing was modelled by the

Monte-Carlo method where the rate of the fast phase was multiplied a factor of $1-\gamma$ for each nearest-neighbour actin site occupied by myosin, a procedure which yields a binding transient not reducible to a sum of exponentials. For the data of Fig. 3D-3F, best fits were achieved with $\gamma = 0.39$.

A1. Trybus, K. and E.W. Taylor. 1980. Kinetic studies of the cooperative binding of subfragment 1 to regulated actin. *Proc. Nat. Acad. Sci. USA* 77:7209-7213.

Appendix B: The energy required to move the pinned chain.

David Smith,
Department of Zoology, La Trobe University, Melbourne, Victoria 3086, Australia.

We require the change in distortion energy of the multiply-pinned chain when one point of the chain is moved to a different angle. This energy can be calculated approximately from Eqn. 1 in terms of the interactions between nearest-neighbour pairs of pinning sites (B1). For two pinning sites separated by distance x, with pinning angles ϕ_1 and ϕ_2, the distortion energy is of the form

$$E_{1,2}^{(2)}(X) = A(\phi_1) + A(\phi_2) + V_{1,2}^{(2)}(X) \tag{B1a}$$

where $X = x/L_P$, $A(\phi) = \phi^2/2\sigma_o^2$ is the energy associated with a single pinning site, and

$$\beta V_{1,2}^{(2)}(X) = \tfrac{1}{2}a(X)\left\{\frac{\phi_1^{\,2}}{\sigma_o^2} + \frac{\phi_2^{\,2}}{\sigma_o^2}\right\} + b(X)\frac{\phi_1\phi_2}{\sigma_o^{\,2}} \tag{B1b}$$

where $\beta = 1/k_BT$. Analytic forms for the coefficient functions are available through the auxiliary functions $\Gamma_S(X)$ and $\Gamma_A(X)$ (B1), where

$$\begin{aligned} a(X) &= \tfrac{1}{4}\{\Gamma_S(X) + \Gamma_A(X) - 4\}, \\ b(X) &= \tfrac{1}{4}\{\Gamma_S(X) - \Gamma_A(X)\}. \end{aligned} \tag{B2}$$

When $X >> 1$, $\Gamma_S(X)$ and $\Gamma_A(X) \to 2$, so $a(X)$, $b(X)$, $V^{(2)}(X) \to 0$ and the distortion energy of the pair is the sum of the one-site energies. When $X << 1$, $\Gamma_S(X) \to 1$ and $\Gamma_A(X) \sim X^{-2}$, so $a(X) + b(X) \to -1/2$. In this limit, the distortion energy of a homopair ($\phi_1=\phi_2$) is the energy from a single pinning site, while that of a heteropair diverges to ∞.

If the occupancy of each actin site by myosin or TnI is known, the effect of chain distortion on their actin affinities can be calculated approximately, as follows. We require the change in chain distortion energy on adding a bound protein which pins the chain at angle ϕ_3, when the chain is pinned at angles ϕ_1,ϕ_2 by nearest-neighbour bound proteins at distances x,y respectively, one on each side. The cost in chain energy is $\Delta E_{1,3,2}(X,Y) = E_{1,3,2}^{(3)}(X,Y) - E_{1,2}^{(2)}(X+Y)$ where

$$E_{1,3,2}^{(3)}(X,Y) = A(\phi_1) + A(\phi_2) + A(\phi_3) + V_{1,3}^{(2)}(X) + V_{3,2}^{(2)}(Y) + V_{1,2}^{(2)}(Y) + V_{1,3,2}^{(3)}(X,Y) \tag{B3a}$$

and $V^{(3)}$ is the triplet interaction energy. If $V^{(3)}$ is neglected, the cost in chain distortion energy is

$$\Delta E_{1,3,2}(X,Y) = A(\phi_3) + V_{1,3}^{(2)}(X) + V_{3,2}^{(2)}(Y) \tag{B3b}$$

which is a quadratic function of the three pinning angles. In this way we find that

$$\beta\Delta E_{1,3,2}(X,Y) = \frac{(\phi_3 - \bar{\phi}_{1,2}(X,Y))^2}{2\sigma_{1,2}(X,Y)^2} + \varepsilon_{1,2}(X,Y) \tag{B4}$$

where

$$\frac{\bar{\phi}_{1,2}(X,Y)}{\sigma_o} = -\frac{b(X)\phi_1 + b(Y)\phi_2}{1 + a(X) + a(Y)}, \tag{B5a}$$

$$\frac{\sigma_{1,2}(X,Y)}{\sigma_o} = (1 + a(X) + a(Y))^{-1/2}. \tag{B5b}$$

The formula for the remainder $\varepsilon_{12}(X,Y)$ is not shown: it is numerically very small, of order $0.01k_BT$ or less. No remainder is expected because the quadratic form for $\Delta E_{1,3,2}(X,Y)$ as a function of ϕ_3 should have a minimum value of zero when ϕ_3 takes its equilibrium value between pinning sites 1 and 2. Thus ε_{12} is a measure of the neglect of the triplet interaction energy. With $\phi_3 = \phi$, $\Delta E_{1,3,2}(X,Y)$ is the quantity $\Delta E_{1,2}(\phi|X,Y)$ defined in the main text.

Exact chain-model calculations with given pinning centres are desirable, but computationally difficult; the mean chain configuration follows by energy minimization, but a finite-temperature calculation is required to calculate standard deviations along the chain.

B1. Smith, D.A. and M.A. Geeves. 2003. Cooperative regulation of myosin-actin interactions by a continuous flexible chain. II: actin-tropomyosin-troponin and regulation by calcium. *Biophys. J.* 84:3168-3180.