A Mechanistic Model of the Motility of Actin Filaments on Myosin

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ABSTRACT

The interaction of actin filaments with myosin is crucial to cell motility, muscular contraction, cell division and other processes. The *in vitro* motility assay involves the motion of actin filaments on a substrate coated with myosin, and is used extensively to investigate the dynamics of the actomyosin system. Following on from previous work, we propose a new mechanical model of actin motility on myosin, wherein a filament is modeled as a chain of beads connected by harmonic springs. This imposes a limitation on the 'stretching' of the filament. The rotation of one bead with respect to its neighbors is also constrained in similar way. We implemented this model and used Monte Carlo simulations to determine whether it can predict the directionality of filament motion. The principal advantages of this model are that we have removed the empirically correct but artificial assumption that the filament moves like a 'worm' i.e. the head determines the direction of movement and the rest of the filament 'follows' the head as well as the inclusion of dependencies on experimental rate constants (and so also on e.g. ATP concentration) via the cross-bridge cycle.

Keywords: Actin, myosin, motility, modeling

1 INTRODUCTION

Myosin is a type of molecular motor that brings about unidirectional movement of actin filaments using the energy produced by the hydrolysis of ATP. Muscular contraction occurs due to the sliding of actin filaments towards the center of bipolar myosin filaments. Additionally, in both muscular and non-muscular cells, the actin-myosin system is responsible for various cellular movement and shape-changing functions.

The *in vitro* motility assay is used to investigate the function of this and other molecular motor systems by arranging for actin filaments to slide along a surface coated with myosin, for myosin-coated microbeads to move along an actin-covered surface, or some variation on the above, in the presence of sufficient ATP. This assay is extensively used in assessing the impact of different chemical species (e.g. potential drugs, toxins, etc.) on the biomolecular motility which is involved in bioprocesses as varied as muscular contraction; neuroprocesses; and cell division.

In previous work [2], we proposed a model for actin motion on a myosin-coated surface that involved a myosin-actin filament power-stroke force that depended on the length of each filament as well as the density of myosin on the model surface. We made the artificial assumption that the "head" monomer of the actin controls the direction of the filament and all other monomers follow the head. Although this very simple model captures extremely well the observed sliding motion of filaments on myosin during motility assays, it is clear that it is inadequate for the purposes of predicting physical characteristics of the system *ab initio*.

In the present paper, we have made several important improvements to the model. Firstly, we have eliminated the assumption of "head controls direction" — instead, we model the filament as a chain of arbitrary-shaped beads connected by harmonic springs. Secondly, we have constrained the motion of the filament laterally, to account for its rigidity (also using a harmonic spring model). We have also introduced dependence upon ATP concentration, temperature and other environmental factors via the integration into the model of a cross-bridge cycle model. This is modeled as a Markov chain and evaluated stochastically over time in a Monte Carlo-like fashion, with arbitrary rate constants for each state transition. The present model begins with the meso-scale interaction of each monomer with a myosin head (rather than treating the filament as a whole) and thus is of a far greater predictive value as well as being physically meaningful. We envisage that it could be used not only for predicting the characteristics of actin-myosin interaction e.g. velocity versus force relationships for molecular motors but also for validation models of motility and thus muscular function as well as other critical biological processes.

2 MOTILITY MODEL

The model consists of three relatively independent components. The first of these is models the myosin molecules geometrically as planar structures equipped with two "arms" for binding to the actin filament. The second models these filaments as chains of beads connected by harmonic springs. Finally, the interaction of a model filament with a model myosin molecule is calculated under the framework of a stochastic Markov chain cross-bridge cycle model. In what follows we briefly describe each of these in turn.

2.1 Myosin Model

Myosin is a large protein molecule that uses chemical energy to perform directional biological motion. Myosin captures a molecule of ATP, the molecule used to transfer energy in cells and breaks it down, using the chemical energy produced to perform the so-called "power stroke" — pushing the actin filament. Myosin is composed of several protein chains: two large "heavy" chains and four small "light" chains and has a complex structure. For the purposes of motility, however, one can model the myosin as a "base" equipped with two "arms" that bind to the monomers in an actin filament and carry out a power-stroke.

Accordingly, the myosin head is modeled as a two-dimensional circle of radius r_{myo} oriented horizontally on an infinite (polymer) plane. A head has two "arms" and these act independently of each other. At any time an arm can be bound to an actin monomer or free. If bound, the actin monomer and myosin arm go through the cross-bridge cycle (see below). More precisely, within the "bound" state several other states exist — each corresponding to a stage of the cross-bridge cycle (the system traverses this cycle stochastically). When this is completed, the myosin arm becomes free to bind to another monomer. Arms can only bind to monomers that are "above" the circle representing the myosin head. The myosins are randomly distributed on the model surface with number density n_{myo}.

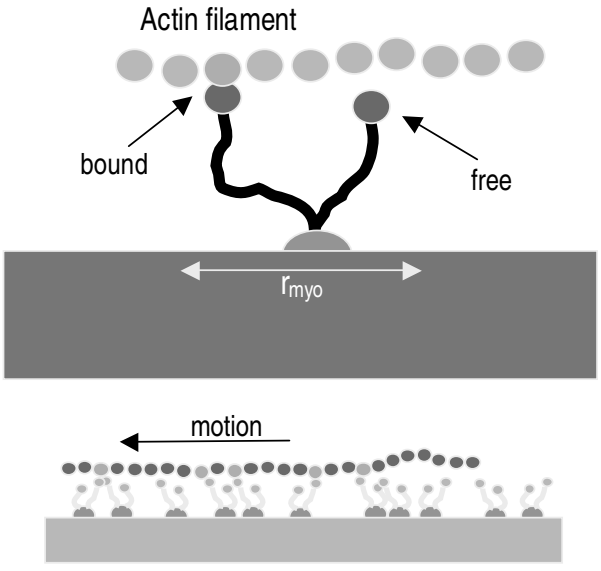

Figure 1: Interaction of an actin filament with a myosin-coated surface.

2.2 Actin Model

Actin filaments are modeled as chains of N arbitrary-shape monomers of mass m and nominal radius r, connected together by harmonic springs. Each monomer is connected to the next by a harmonic spring linking their centers in two dimensions (we assume the filament to remain horizontal and equidistant from the myosin-coated surface and thus ignore the z dimension) along the direction of an inbuilt "axis", $\mathbf{a}_i$ for the i^{th} filament. All springs are identical with rest length l_{rest} and spring constant k. The force exerted on a monomer i from the two adjacent monomers is then given by

$$F_{spring}(i) = -k\left(\left|\left(\mathbf{p_{i-1}} - \mathbf{p_i}\right)\right| + \left|\left(\mathbf{p_{i+1}} - \mathbf{p_i}\right)\right| - 2l_{rest}\right) \quad (1)$$

where $\mathbf{p_i}$, $\mathbf{p_{i+1}}$ and $\mathbf{p_{i-1}}$ are the position vectors of monomers i, i+1 and i-1, respectively in 2-space.

Monomers are also constrained laterally. Let the angle between monomers i-1, i and i+1 be $\alpha_i(t)$ and let the straight line connecting monomers i-1 and i+1 be L. Then monomer i experiences a force proportional to $(\pi\text{-}\alpha_i(t))$ in the direction of a vector between the center of monomer i and the midpoint of L. The constant of proportionality is k_α. This then gives the angular response as

$$\frac{\partial^2 \alpha_i(t)}{\partial t^2} = -k_\alpha\left(\pi - \alpha_i(t)\right) \quad (2)$$

It is often the case that the behavior of the bonds connecting monomers in a polymer molecule is assumed to be harmonic. In this case we have also made this assumption for both types of response (positional and angular). It is probably safe to say that this is a good first approximation — however, this may be changed in a future model, depending on how well the present model captures the characteristics of a motility assay (and thus of the actin-myosin interaction in general).

Monomers experience three other forces. The first and most crucial of these is due to the power-stroke from the myosin arm. While the system is in the power-stroke state, the monomer that is bound to the myosin arm experiences a constant force, F_{myo}. This force is exerted in the direction of the monomer's axis — this assumption is physically meaningful since the binding of the myosin arm to the actin filament only happens in certain orientations of these and hence the power-stroke can only be applied in a given direction (possibly slightly perturbed due to thermal forces).

A frictional force proportional to the velocity of each monomer $|\mathbf{v}_i|$, with proportionality constant γ and opposed to the velocity vector is also experienced. This constant can either be estimated empirically or calculated under certain assumptions about the shape of each monomer (the assumption that monomers are spherical thus leads to the

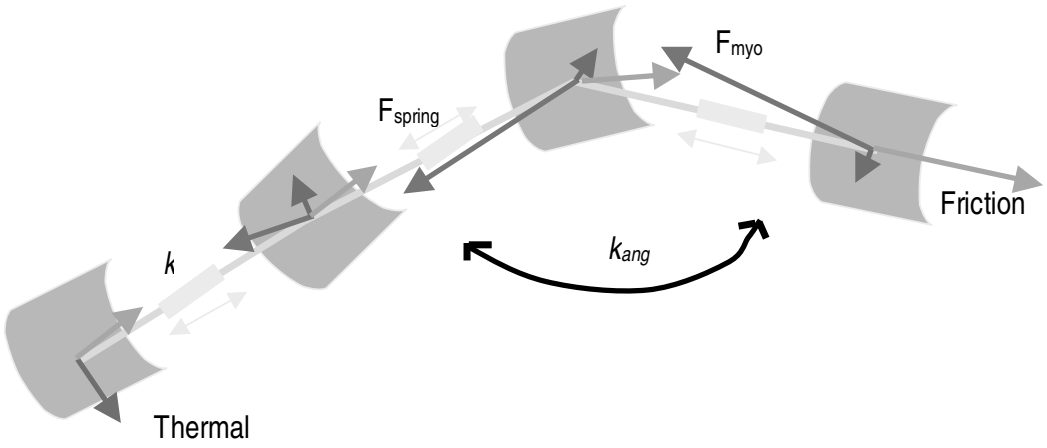

Figure 2: Mechanical model of actin filament.

Stokes' law constant). Finally, each monomer experiences a thermal force of normal strength (where the maximal strength depends on absolute temperature) distribution and random direction at each point in time. The sum of these forces on each monomer gives the total force experienced. At each time step, these are recomputed. The time step has length Δt.

2.3 Cross-Bridge Cycle Model

In order to model the interaction of a myosin head with an actin monomer in a physically meaningful way, we have introduced into our model a stochastic Markov chain cross-bridge cycle. The widely accepted scheme for this cycle was proposed by Lymn and Taylor [4] and is used here.

Initially, the myosin cross-bridge is unbound — in our model, the probability of binding at any time is $2p$ if a monomer is within the action radius of a myosin molecule and both arms are unbound, p if only one arm is bound and 0 if both arms are bound or no monomer is within the action radius. Upon binding, the myosin cross-bridge enters the weakly bound (non-force-producing) (A)·M·ADP·P_i state (A and M denote actin and myosin, respectively and the brackets indicate weak binding). Releasing the phosphate P_i leads to the next state — the strongly bound, force-producing A·M*·ADP state, leading to the execution of the power stroke. In our model, the length of this power stroke is a random variable which depends upon the rate constant for the transition to the next state. During the power stroke the monomer experiences a force F_{myo} along its axis direction. Thus the power stroke produces conformational changes in the myosin molecule and a translation of the monomer (and at a larger scale, the filament). The resultant A·M·ADP state can no longer bind P_i and at the end of the stroke, ADP is released and the system enters the A·M state.

If ADP rebinds, then the system will continue to resist both motion and attachment. Eventually, ATP binds, leading to a detachment of the cross-bridge. Upon hydrolysis the cross-bridge enters the (A)·M·ADP·P_i state and can thus participate in a new working stroke [4]. The transition from each state to the next is controlled by the rate constants, which for different systems can be found in the literature and depend on ATP concentration, temperature and other factors. On the other hand, these can be estimated if other parameters (e.g. force) as well as motility characteristics are known in advance.

3 SIMULATION RESULTS

We have implemented our model into an in-house developed simulation program to facilitate the development and validation of the model. This program can be used to specify parameters (e.g. rate constants, filament length etc.) and extract simulation descriptors (e.g. filament velocity) under different conditions.

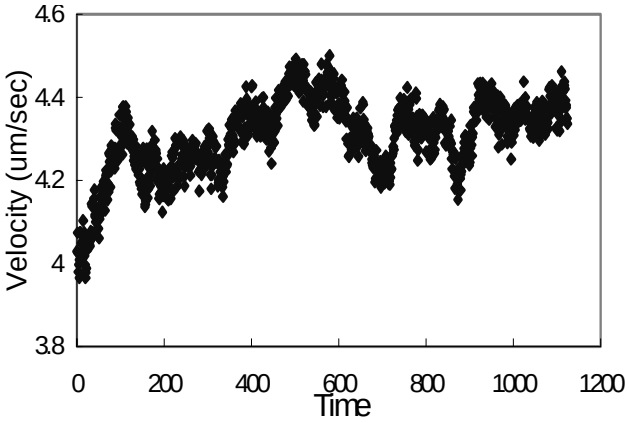

Figure 3: Velocity versus time in a typical simulation.

4 DISCUSSION

The motility of the protein molecular motors has been used in primitive dynamic nanodevices that have enormous potential in sensing, nano-level power generation and possibly computation [3]. It is difficult, however, to probe the feasibility of the usage of protein molecular motors in "real-device" conditions, that is, far from the conditions present in carefully optimized motility assays; thus, the simulation of motility assays with phenomenologically-relevant input parameters would constitute a very useful tool.

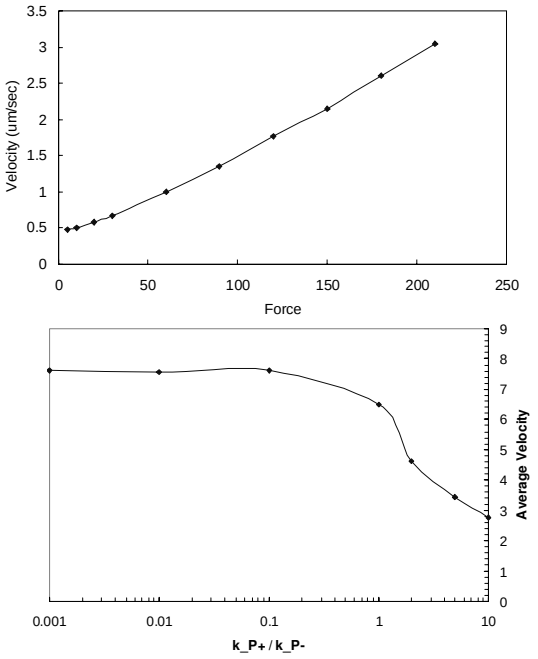

Figure 4. Velocity versus force (top) and forward/backwards rate constant ratio (bottom).

Biological molecular motors are generally thought to "rectify" Brownian motion — i.e. operate via a "Brownian ratchet" mechanism. The model proposed here also relies on a such an interaction between the actin filament and the myosin motor. The directionality of the motion (as shown by simulations) is guaranteed by the in-built orientation of actin monomers in a filament and the geometrical specificity of the myosin-actin binding. Finally, the quantitative nature of the interaction between the motor and the filament is controlled by the hydrolysis cycle rate constants. The qualitatively correct simulation results suggest that a Brownian ratchet mechanism combined with a stochastic hydrolysis cycle model can predict the nature (especially directionality) of the actin motility.

The combination of three models, while making for a fairly comprehensive overall description of actin-myosin based motility comes at the price of complexity — the model requires around 20 parameters (around 6 geometrical constants, 8 rate constants and 6 force/flexibility constants). Consequently, it cannot be considered analytically determinate in a practical sense — i.e. in order to predict one of the parameters (e.g. actin flexibility), it is necessary to estimate the others. For some parameters, e.g. filament-fluid drag coefficient, this can be done theoretically, while for others, e.g. rate constants, experimental data is available. The fully quantitative validation of the model using physical parameters is the subject of future work.

5 CONCLUSION

A comprehensive model for the motility of actin filaments on a myosin-coated substrate has been presented, comprising separate models of the hydrolysis cycle and mechanics of actin filaments and myosin motors. We have shown via simulation that the model is capable of predicting qualitatively the motility of actin filaments (especially directionality) and produces empirically credible relationships between parameters and observed motility descriptors (e.g. velocity). Further work will concentrate on the fully numerical evaluation of simulation results using empirically estimated biomolecular parameters.

6 ACKNOWLEDGMENTS

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