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A coarse-grained molecular model for actin–myosin simulation

William R. Taylor*, Zoe Katsimitsoulia

Division of Mathematical Biology, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

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ABSTRACT

We describe a very coarse-grained molecular model for the simulation of myosin V on an actin filament. The molecular representation is hierarchical with the finest level representing secondary structure elements (end-points) which are grouped into domains which are then grouped into molecules. Each level moves with a Brownian-like motion both in translation and rotation. Molecular integrity is maintained by steric exclusion and inter-domain restraints. A molecular description is developed for a myosin dimer on a actin filament with binding interactions also specified between domains to simulate both loose and tight binding. The stability of the model was tested in the pre- and post-power-stroke conformations with simulations in both states being used to test the preferred binding site of the myosin on the filament. The effects of the myosin twofold symmetry and the restriction of an attached cargo were also tested. These results provide the basis for the development of a dynamic model of processive motion.

1. Introduction

Motion within the eukaryotic cell is based on a protein motor moving along a polymer track. In some situations, the polymer might be nucleic acid and the motor a polymerase or a helicase or even the ribosome but more generally, cell motility employs either kinesin or dynein moving along a microtubule (an α/β tubulin polymer) or myosin on an actin filament (an F-actin polymer). With their employment in muscle, the actin–myosin system is by far the most abundant motor in higher organisms but it also has great diversity, as is reflected in the many different sub-types of myosin. For example, actin–myosin motors are employed for the transport of vesicles inside the cell using double-headed motors or with motors arranged in arrays they can power large scale motion, as seen in muscle or in the invasion of the red-blood by the malarial parasite. The former type of motion, in which the motor undergoes multiple chemical cycles before detaching, is referred to as processive, in contrast to the motion of filaments along a bank of static motors [1–4].

The operation of muscle myosin (myosin-II) has been extensively studied over many years and more recently, X-ray and electron based structures have been solved for almost every stage in the myosin motive cycle. Processive myosins typically transport cargo inside the cell, either forward (e.g. myosin-V) or backwards (myosin-VI) relative to the actin filament direction and are less well studied, with X-ray structures having only recently been solved. While these structures show the details of the molecule, they indicate little about the coordination of motion (if any) between the paired motors. Information on this aspect comes either from biophysical studies or electron microscopy. The latter can provide snap-shots but no dynamics while the former can provide information on dynamics but without atomic scale visualisation.

All studies point to an ATP-driven mechanical cycle common to all myosin motors. This, arbitrarily, begins with the ATP bound myosin free of actin. With ATP hydrolysis, the myosin can bind to actin but retains the hydrolysis products, ADP and inorganic phosphate (Pi). With the release of Pi, the myosin undergoes a large conformation change associated with motion, called the "power-stroke" in which the main catalytic and actin binding domain swings through a large angle relative to its fixed point of attachment at the opposite end of the molecule. This tight-binding conformation, called the "rigor" state, is retained until ADP is released and with ATP binding, the myosin dissociates from actin and the cycle repeats. This cycle is shown in the context of the two chains of a myosin-V dimer in Fig. 1.

An approach to synthesise the structural data with kinetic data is through a molecular model. However, a double-motor myosin, with a track of actin to run along, constitutes a very large system which would be far too big to simulate at the atomic level using molecular dynamics, except perhaps on the worlds largest supercomputers. This has led to the development of course-grained (CG) models in which groups of atoms, or residues, are represented by a single point. Through such reductions it is hoped that simulations of a useful length can be run on moderate computers without having reduced the molecular representation to a level at which high-resolution structural information has been lost [5–7].

In this work we develop a multi-level CG molecular model for myosin-V on actin that employs a hierarchy of three structural lev-

* Corresponding author. Tel.: +44 208 8162298; fax: +44 208 8182460.
E-mail address: wtaylor@nimr.mrc.ac.uk (W.R. Taylor).

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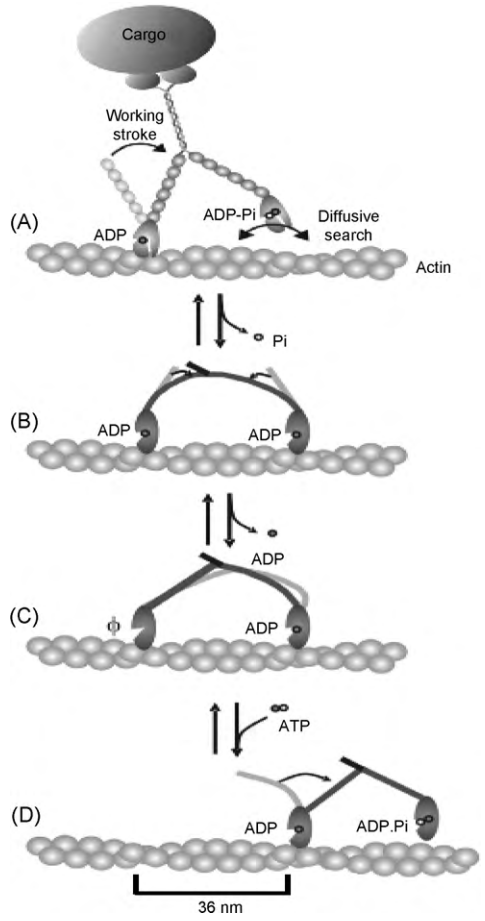

Fig. 1. Myosin-V ATP cycle. (a) With release of Pi, the left leg makes a power stroke allowing the right leg to search for an actin binding site. (b) The bound right leg binds and loses its Pi, creating strain in the legs (arrows). (c) With loss of ADP in the left leg, the power stroke can progress in the right leg. (d) On ATP binding, the left leg is released from actin and swings to the right and with ATP hydrolysis, it to returns to the pre-power-stroke conformation. (Reproduced from [21], with permission.)

els. At the lowest level, each secondary structure element (SSE) is represented as a cylinder. These are then grouped into domains which, in turn, are grouped into individual proteins. Elements at all levels move with a simple Brownian-like motion and collide with each other, however, lower-level elements do not interact between groups unless their parent groups have collided. This hierarchy of interaction is applied recursively across all the proteins in the simulation using the efficient collision-detection algorithm in the graphical programming language Java3D [8].

2. Methods

2.1. Myosin model

The molecular structure of myosin-V (PDB code: 2DFS [9]) consists of two heavy-chains, of over 1000 residues each, linked by a 130 residue length of coiled-coil at their carboxy-terminal ends (Fig. 2(a)). The globular amino-terminal motor domain extends into a long α-helix which, at regular intervals, is bound by six calmodulin-like light-chains (Fig. 2(b)). This region consists of repeated binding sites called "IQ" motifs to which the light-chains bind. The molecular structure shown in Fig. 2 is in the "resting" state where the carboxy terminus is bound back to the amino terminus (by unresolved domains) preventing any motion. When cargo is bound to the carboxy-terminal coiled-coil, the motor domains become free to bind to actin and begin moving. Excluding the coiled-coil segment, the two heavy-chains (referred to below as "legs") have a close, but not exact twofold symmetry. In anthropomorphic terms, the molecule must therefore walk having two left (or right) legs and, given that each globular motor or "foot" domain,[1] binds actin with the same orientation, then some interesting gymnastics must result.

Taking the coordinates of the myosin-V structure (2DFS), secondary structure elements (SSEs) were defined using the `stick` program that identifies all linear segment in a protein α-carbon backbone and establishes their end-points based on the minimum moment of inertia about each line, summed over all lines [10]. This method is not sensitive to details of hydrogen-bonding and can provide robust secondary structure definitions on models and low-resolution structures, such as 2DFS which has a nominal resolution of 24 Å (Fig. 2(c) and (d)).

2.1.1. Domain assignment in the 'foot'

The foot-domain (residues 1–770 in 2DFS) was analysed for sub-domains that could form the basis of a structural hierarchy. However, an automatic method [11] with default settings split the molecule only into two parts. The single parameter in this method that controls the granularity of the domains (`spread`) was reduced to 10, generating the finer division into six domains shown in Fig. 3. This separated the core β/α ATPase domain of the molecule along with three domains that form the ATP-binding cleft, two of which interact with actin, and two domains, including the all-β SH3 domain at the N-terminus and the mainly-α domain at the C-terminus that leads into the long light-chain binding helix. The latter two domains are central to the leg/foot bending motion of the molecule and will be referred to jointly as "ankle" domains. These domains correspond reasonably with those identified originally by 'eye' [12] and have some correspondence to units identified by normal mode analysis on myosin-II [13].

Five of the six domains range from 50 to 100 residues with the core β/α domain over 200 residues. A similar distribution of sizes was also seen in the number of SSEs and, although a structural hierarchy in our simple Brownian dynamics method [8] can be imbalanced, for computational reasons, we preferred that each level in the hierarchy should contain an equal number of between 10 and 20 components. To equalise the size of the domains at the secondary structure level, some small secondary structure elements were removed from the fringes and the core domain was split into two halves. After some 'trading' of secondary structure between adjacent domains, seven domains were defined with six secondary structure elements each and, with some exceptions, these largely preserve their order in the chain and correspond well to the domains defined automatically at the α-carbon level (Fig. 3).

As the myosin foot domains (numbered 1–7) will be referred to frequently, codes that reflect their function were assigned as: the "ankle" domains A1, A2 (1 and 7), the "binding" domains B1, B2 (5 and 6) and the "core" domains C1, C2 and C3 (2, 3 and 4) (Fig. 3(f)). In the terminology commonly used in the muscle literature, these domains correspond as: A1 = SH3, A2 = converter, B1 + C2 + C3 = upper 50 K, B2 + C1 = lower 50 K. The centroids of these domains were then used to define a coordinate reference frame for the foot with the vector in the direction C1 → C3 in the *X* direction, the vector in the direction B2 → C2 in the *Y* direction, the *Z*-axis was defined as orthogonal to *X* and *Y* and the *Y*-axis redefined as orthogonal to *X* and *Z* giving a set of three mutually orthogonal axes forming a right-hand coordinate reference frame. Unit vectors

[1] The parts we refer to here as "leg" and "foot" are more commonly referred to in the muscle literature as "arm" and "head", respectively. However, with the obvious walking behaviour of myosin-V, we will adhere to the names of body parts that are more closely associated with locomotion.