Review of: "High-resolution structures of the actomyosin-V complex in three nucleotide states provide insights into the force generation mechanism"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

General Comments

This is a large and complex Ms. What is new is the structure of myosin complexed with AppNHP and interacting with actin as seen by cryo-EM. The result is not very different from myosin-ADP-actin complex, and maybe on the way to ADP dissociation to form the AM complex. I assume that experiments were done correctly and so were the analyses. There are several places where the contents should be made clearer, and speculations must be stated as such.

Xray crystallography has made a significant advance in the last 30 years, staring from the structure of actin [1] and myosin [2]. Combined with cryo-EM, the interaction of actin and myosin, and the structure of the thin filament with/without regulatory proteins Tpm/Tn have made a significant advance, and one can now make simulations using molecular dynamics to demonstrate the details of movements of individual arms and residues of contractile proteins, mostly of myosin. These are, however, educated speculations, and there always is a possibility that these models may not be real. That is where experimental evidence comes in, and models thus created must be consistent with the experimental observations.

Your arguments (lines 59-61, 617-629, and Fig 11: power stroke after Pi release) are interesting. However, these are speculations based on crystallographic structures, and they contradict to the observation that Pi release occurs after force is generated based on skinned fiber experiments [3-5]. Limitations of these structural studies include: (1) force cannot be measured in crystals or cryo-EM, (2) actin is absent (even present in cryo-EM structures, myosin structure used is based on the crystals made in the absence of actin), (3) Tpm and Tn are absent, and (4) crystals were not created under the physiological ionic conditions. This reviewer understands that these are technically challenging and may not be achievable, however, a reflection may be necessary where your model is not supported by experiments. It has been known in the last 50 years that in the absence of actin, myosin ATPase is very low [6]. In the presence of actin, ATPase increases significantly [6] and force is generated, hence there must be a large change in the myosin conformation. Even when actin is present, Tpm (and Tn) is known to double the ATP hydrolysis rate [7-9] and force generation [10], hence myosin structure must further change significantly when Tpm and Tn are present. In addition, there is a big difference in experimental conditions, such as ionic strength

and protein concentrations. Until these problems are solved, you must recognize that your molecular mechanism is a hypothesis or speculation, and this fact must be clearly stated. Since biology is an experiments-based science, one should respect experimental evidence. These facts do not undervalue your work. In fact, the data quality is extremely high, and mechanisms proposed are intriguing. However, you must not forget limitations of your approach, and recognize that your models (molecular mechanisms of force generation) in places are not supported by actual force measurements such as by [3-5]. Because the latter is based on force measurements carried out under the physiological solutions, this disparity is important and must be recognized.

Specific comments

There must be a table of abbreviations. They include (but not limited to):

PHD: phalloidin

PPS: Pre power stroke

PRT: post-rigor transition (state)

PiR: phosphate release

PCA: principal component analysis

PPS: pre-power stroke state

Intermediate state: intermediate recovery stroke state (lines 589-590)

PTB: protein data base(?)

L50: lower 50K domain

U50: upper 50K domain

AppNHp à strongly bound post-rigor transition (PRT) state -- reminiscent of the rigor state

The use of “young” and “aged” is not clearly defined. I assume they refer to the state of actin, but in places they appear to refer to the state of myosin (eg: young rigor, aged rigor).

For “young”, I found the followings:

young ATP/ADP-Pi-bound F-actin

young ADP-Pi-bound F-actin

young PHD-stabilized F-actin

young JASP-stabilized F-actin

young F-actin-JASP

young F-actin

young F-actin and rigor

young rigor

young JASP-stabilized actomyosin-V

young JASP-stabilized actomyosin complex

young actomyosin-V

young actomyosin-V complex

young actomyosin complex

Similarly, for "aged", I found the followings:

aged ADP-bound F-actin

aged F-actin-PHD

aged PHD-stabilized F-actin

aged actomyosin-V (complex)

aged PHD-stabilized actomyosin-V

aged conformation of F-actin

aged rigor, ADP and AppNHp

However, how much these are the same, and how much of these are different must be defined, and if the same, terms should be unified. When you say "young ATP/ADP-Pi-bound F-actin", I assume ATP or ADP-Pi is in myosin's nucleotide binding pocket and not in actin, and this must be stated.

Why do you use **"**AppNHP"? Classically, "AMP-PNP" has been used, which implies its structure. AppNHP does not imply the structure. It seems AppNHp bound myosin and ADP bound myosin have very similar in structure. Then what is the significance of introducing AppNHp?

Lines 48, 211, 247, 503 and other places: U50 domain à U50K domain, define that U50K means "upper 50K domain" on its first appearance

211: L50 à lower 50K (same as above)

62-63: smaller second swing is not proven in physiological solutions using skinned fibers [11]. Note a big difference in the experimental conditions (low ionic strength, low protein concentrations) for in vitro motility assays.

75-76: Due to the reluctance of F-actin to crystallize à Because actin bound myosin is not crystalized yet. "resistance" sounds like there is an intention in molecules.

215: HF helix à define

219-223: Switch II (T170-D437) à cannot have so many aa residues.

306-307: Clarify: "suggesting that AppNHp is only weakly bound, compatible with strong F-actin binding" à Isn't the ADP state strongly bound?

310-311: "AppNHp and ADP can both weakly bind to myosin in a conformation reminiscent of the rigor" à must clarify, because there is no nucleotide in rigor.

318-319: Not limited to à do you mean not an artefact?

327: myosin-V-AppNHp strongly bound to F-actin in the PRT state à Do you mean "myosin-V is strongly bound to F-actin while myosin-V weakly coordinates AppNHp"?

407: PTB à spell out (protein data base?)

409: loops known for their flexibility à which loops are you talking about?

418: any other state of myosin à do you mean Myosin-ADP and Myosin-AppNHp states, or more?

452-453: phosphate release à phosphate release from F-actin

460: PPS and P_iR state à define

483, 487: binding pocket (of pyrene to F-actin) à define this carefully. Else confusing with the binding pocket of ATP in myosin; use other term if possible, such as “binding site of pyrene in F-actin”.

495: 18 high-resolution (< 3.7 A) structures à what is the lower limit?

497-516: What causes the conformational heterogeneity of myosin-actin interface (other than L50K of myosin)? Is it because conformation is changing with time, or is it based on thermal fluctuation?

513-514: PC à What is principal component analysis? Must define.

540: What is PC 1–PC 2 space? à Must define

546-547: its coupling seems to be statistical in nature à What do you mean?

592-593: Are you saying myosin-V and myosin-VI are conserved or not conserved?

599-601. The use of “PRT state” and “post rigor state” is strange.

614, 622: You said earlier that the actin binding cleft is closed after ATP binding. Why then it is only “partially closed” at PPS and closes with Pi release?

818 (…, respectively?)

References to this review

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