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How the Load and the Nucleotide State Affect the Actin Filament Binding Mode of the Molecular Motor Myosin V

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The interaction between actin and myosin V has been probed by measuring the unbinding force of individual actomyosin complexes using optical tweezers. Surprisingly, we found that in both the nucleotide-free and ADP-bound states single- and double-headed binding occurs with approximately the same probability. Estimation of the spring constant of individual actomyosin complexes confirmed that in each of the nucleotide states two distinct populations exist. These results confirm that optical nanometry can be used to reliably study the mechanism of how cytoskeleton molecular motors interact with their associated polymer lattices under solution conditions more closely resembling the intracellular environment.

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I. INTRODUCTION

Myosin V is an actin-based molecular motor, which moves unidirectionally along an actin track using two identical catalytic motor domains, called "heads," that performs three main actions: binds to actin, binds and hydrolyzes ATP and transforms the chemical energy of hydrolysis into a large swing of the regulatory domain, which acts as a long lever arm that generates mechanical displacement. The two heads work sequentially, such that at any moment at least one of the heads is bound to actin, which allows the motor to "walk" as individual molecules, in a "hand-over-hand" fashion [1]. Similarly to another type of molecular motor, kinesin, myosin V can adjust to applied external forces [2–5], optimizing its movement through the dense mesh of cytoskeletal filaments, allowing efficient transport of cargo in a crowded cellular environment.

To elucidate the force-dependent regulation of myosin V function, both (a) the molecular conformation in each nucleotide state and (b) the nucleotide state-dependent response of the molecular motor to external force must be characterized. Although structural and biochemical solution studies provide valuable information about myosin V stepping [6], clarification of the dynamic response to external force requires different experimental techniques. Here we addressed this problem by directly applying force to single actin-myosin V complexes, using optical tweezers to measure the unbinding force. We characterized the binding mode of myosin to actin in two nucleotide states (nucleotide-free or ADP-bound; see [7], which is complementary to this paper) and evaluated the stability and the elasticity of an actomyosin bond in both nucleotide states under applied force. This method provides information on both (a) and (b) under solution conditions, in contrast to electron microscopy experiments, which can be complicated by surface adsorption effects.

II. RESULTS AND DISCUSSION

Preparation of proteins. Myosin V was purified from chick brain [8]. The motor domain of myosin V

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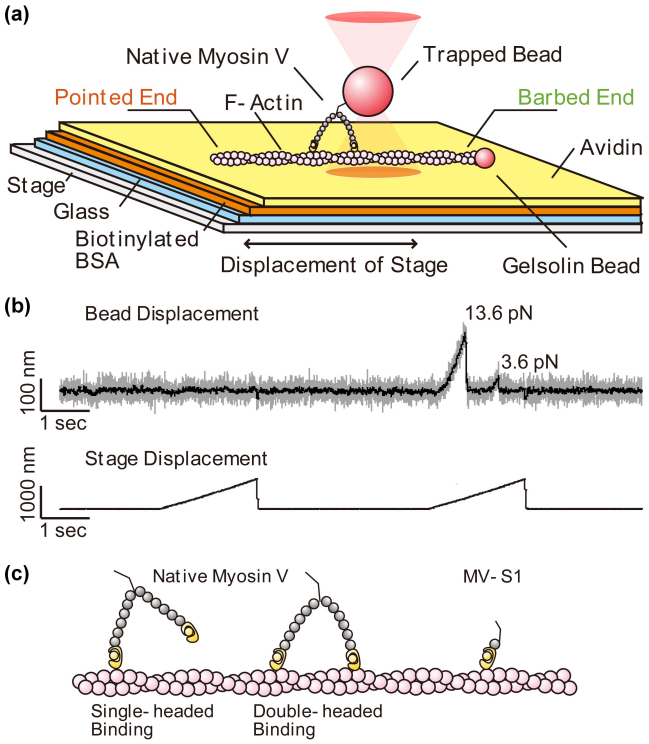

Fig. 1. (a) Schematic illustration of the experimental system. A plastic bead (diameter: 1 μm) to which a single myosin molecule (native myosin V or single-headed MV-S1) is attached is held with optical tweezers and brought close to a 10 %-biotynilated actin filament, immobilized on a glass surface via the biotin-avidin interaction. The polarity of the actin filament was determined using a gelsolin-coated bead (diameter: 0.2 μm), which specifically attaches to its barbed end. (b) A typical trace of bead displacement for native myosin V in the nucleotide-free state. Both the double- and the single-headed interactions were detected during the same pulling event. (c) Schematic representation of the binding modes of native myosin V and of the MV-S1 construct.

containing the first IQ motif (MV-S1) and the essential light chain, LC-1sa, were co-expressed in Sf9 insect cells and purified by FLAG affinity chromatography [9]. Actin purified from rabbit skeletal muscle [10] was 10 %-biotinylated and, after polymerization, labeled with rhodamine phalloidin.

Unbinding force measurements. We measured the force required to break individual actin and myosin V bonds (*i.e.*, the unbinding force) in various nucleotide states using optical tweezers [11–14]. The experiments were performed in assay buffer containing (in mM) 20 HEPES-KOH (pH 7.8), 25 KCl, 5 $MgCl_2$, 1 EGTA. The experimental system was created by consecutive infusion of the following solutions into a flow cell in the order: 3.5 mg ml^{-1} biotinylated BSA (incubated for 4 min), 0.5 mg ml^{-1} streptavidin (incubated for 2 min), fluorescent and polarity-marked 10 %-biotinylated F-actin (incubated for 10 min). The flow cell was washed with two volumes of the assay buffer after each step and, finally, filled with the solution containing myosin-bound beads, oxygen scavenging system (4.5 mg ml^{-1} glucose, 50 U ml^{-1} catalase, 50 U ml^{-1} glucose oxidase, 10 mM DTT) and, when necessary, ADP to a final concentration of 1 mM. For measurements in the presence of ADP, 1 U ml^{-1} hexokinase was added. The experiments were performed at 25 $\pm$ 1 °C. Dimeric molecules of native myosin V were attached to plastic beads at concentrations sufficiently low for observing single actomyosin binding events. A bead was then trapped with an infrared laser ($\lambda = 1064$ nm; trap stiffness: 0.076 pN/nm) and brought close to an actin filament immobilized on the glass surface [4] (Figure 1(a)). The microscope stage was slowly displaced with a constant speed (500 nm/s) in the direction parallel to the actin filament axis, such that its pointed end was leading; therefore, as soon as the actomyosin bond had formed, the displacement of stage gradually increased load on it, directed towards the barbed end of actin, until the bond ruptured and the bead returned to the trap center (Figure 1(b)). The value of load at which the bond breaks was defined as the unbinding force and it was measured both in the absence of nucleotides (nucleotide-free, or the so-called "rigor" state) and at saturating ADP concentration (1 mM; the ADP-bound state). Similar experiments were performed with the genetically modified construct of myosin V (MV-S1), in which the dimer-forming coiled-coiled domain had been removed to ensure that it exists in a monomeric form only (Figure 1(c)).

The distributions of the unbinding forces in the nucleotide-free state measured for the dimeric native myosin V are bimodal, with two peaks corresponding to weak (4.3 pN, determined from the Gaussian fit, W-component) and strong (13.6 pN, S-component) unbinding forces (Figure 2(a)). This observation suggests that native, double-headed myosin V binds actin in two distinct binding modes. Single-headed myosin V (MV-S1) displays a single peak distribution and binding mode (Figure 2(b)), positioned similarly to the W-peak in the bimodal distribution (4.2 pN). It strongly suggests that the W-component of the bimodal distribution corresponds to single-headed binding and the S-component thus corresponds to double-headed binding. Interestingly, the value of the S-peak is ~3-fold larger than that of the W-peak, indicating that force required to rupture the double-headed binding exceeds the estimated value of force necessary to unbind two single-headed molecules bound in parallel ($4.2 \times 2 = 8.4$ pN). The necessity to exert an additional ~5 pN to rupture double-headed binding of native myosin V, compared with two single-headed molecules, hints at a possible communication between the two bound heads in the dimeric molecule, which results in a stronger binding of a dimer than that of two monomers; another possibility is that the presence of the second head changes the manner of the load exertion, reducing the effective load on the actomyosin binding interface and requiring a larger force to rupture the ac-

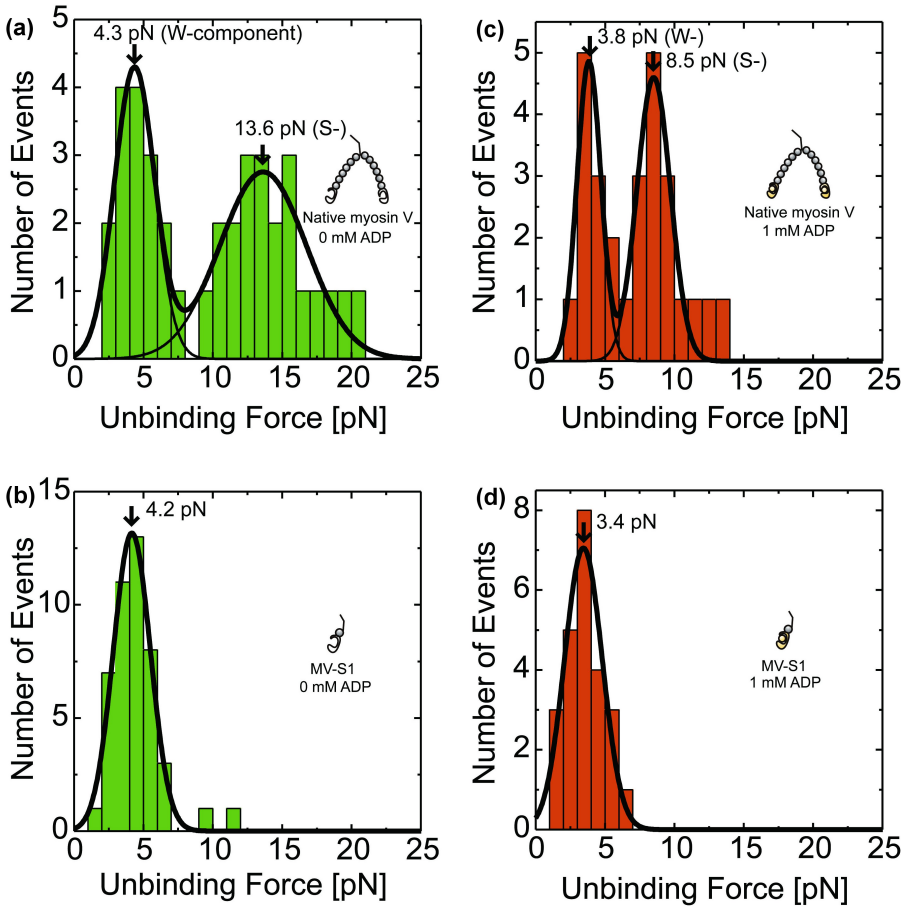

Fig. 2. Unbinding force distributions for native myosin V in the nucleotide-free (a) and the ADP-bound state (c) and for single-headed MV-S1 in the nucleotide-free (b) and the ADP-bound state (d).

tomyosin bond.

Next, we measured the unbinding forces in the presence of 1 mM ADP. Similarly to the nucleotide-free conditions, the native myosin V distribution exhibited two peaks (3.8 pN for the W-component and 8.5 pN for the S-component) (Figure 2(c)), while a single peak at 3.4 pN was observed with the single-headed MV-S1 (Figure 2(d)). Applying the same interpretation as for the nucleotide-free state, the W-component of the bimodal distribution corresponds to single-headed binding, while the S-component indicates double-headed binding; in the ADP-bound state, similarly to the nucleotide-free state, both single- and double-headed binding occur. However, the absolute value of the W-component of the distributions, as well as that of the single peak of MV-S1, is smaller in the ADP state than in the nucleotide-free state, indicating that the binding of ADP decreases the stability of the actomyosin V bond. Interestingly, the absolute value of the S-component only slightly exceeds twice that of the W-component, unlike the nucleotide-free conditions. This may indicate that ADP binding decreases head-to-head communication, making the two heads of a dimer work more like two separate, independent heads; another possibility is that the ADP-bound head is more adaptive to external load than the head without a bound nucleotide, thus the manner of how load is exerted on the heads is largely the same in both the single- and the double-headed molecules. Judging from the area of the W- and the S-components in both the nucleotide-free and the ADP-bound states, we conclude that dimeric myosin V binds actin with both heads at least as easy as with a single head, which contradicts the conclusion based on the electron microscopy data [6] that the two-headed binding of myosin V to actin is structurally unfavorable in the absence of nucleotides, unlike the ADP-bound state.

In addition to the absolute values of the unbinding force, the raw traces, as shown in Figure 1(b), also provide information on the relation between the applied load and the extension of the actomyosin complex (Figure 3(a)). As this relation is almost linear, the elasticity of the actomyosin complex can thus be estimated (note that the spring constant thus obtained does not contain the contribution from that of actin filaments, because it must be at least an order of magnitude larger). The spring constant plotted against the unbinding force

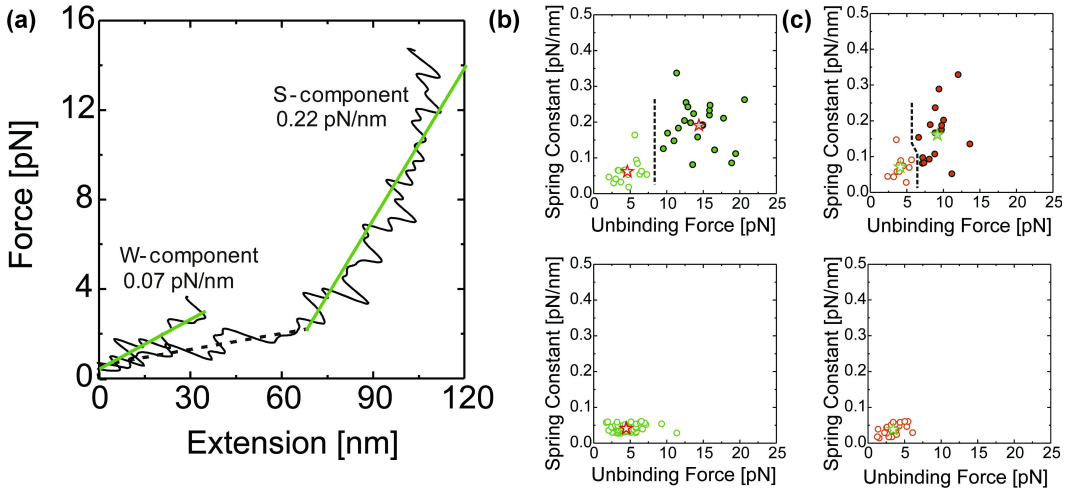

Fig. 3. (a) Force-extension relationship for the W- and the S-populations. (b) Spring constant plotted against the unbinding force for native myosin V (top) or single-headed MV-S1 (bottom) in the nucleotide-free state. (c) Spring constant plotted against the unbinding force for native myosin V (top) or single-headed MV-S1 (bottom) in the ADP-bound state. In both (b) and (c), the open and the closed symbols correspond to the W- and the S-populations, respectively. The star marks indicate the positions of the centers of mass of the corresponding populations (see Table 1 for the numerical values).

Table 1. Unbinding forces and spring constants of W- and S-populations for native myosin V and MV-S1 construct complexed with actin, under directional loading towards the barbed end of actin (average ± S.E.M.).

	Unbinding force [pN]				Spring constant [pN/nm]			
	Nucleotide-free		ADP		Nucleotide-free		ADP	
	W-	S-	W-	S-	W-	S-	W-	S-
Native	4.6 ± 0.4 ($n = 16$)	14.4 ± 0.7 ($n = 21$)	4.1 ± 0.3 ($n = 11$)	9.2 ± 0.5 ($n = 16$)	0.062 ± 0.008 ($n = 16$)	0.2 ± 0.014 ($n = 21$)	0.071 ± 0.01 ($n = 11$)	0.161 ± 0.02 ($n = 16$)
MV-S1	4.4 ± 0.3 ($n = 51$)	-	3.4 ± 0.3 ($n = 24$)	-	0.042 ± 0.001 ($n = 51$)	-	0.039 ± 0.003 ($n = 24$)	-

showed two different populations in both the nucleotide-free (Figure 3(b, top)) and the ADP-bound (Figure 3(c, top)) states. The average unbinding forces for these two populations, summarized in Table 1, correlate well with the values of the two peaks in the Gaussian distributions (Figure 2(a,c)), confirming that they correspond to the W- and S-components. The analysis of the data revealed that in both nucleotide states the stiffness of the actomyosin complex in the S-population exceeds that of the W-population more than two-fold (Table 1), which confirms that the W-to-S transition is induced by the binding of the second head.

III. CONCLUSION

By measuring the unbinding force of individual actomyosin complexes in the nucleotide-free and the ADP-bound states we found that in both nucleotide states single- and double-headed binding of myosin V to actin occurs with approximately the same probability. Similar measurements may be performed in other nucleotide states as well, as we previously did with kinesin [14]. Coupled with the possibility to reveal the force-dependence of nucleotide binding, this method therefore provides an efficient tool to study the mechanism of how the molecular motors work, under the conditions similar to their in vivo environment.

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REFERENCES

[1] A. Yildiz, J. N. Forkey, S. A. McKinney, T. Ha, Y. E. Goldman and P. R. Selvin, Science **300**, 2061 (2003).
[2] A. D. Mehta, R. S. Rock, M. Rief, S. A. Spudich, M. S. Mooseker and R. E. Cheney, Nature **400**, 590 (1999).
[3] M. Rief, R. S. Rock, A. D. Mehta, M. S. Mooseker, R. E. Cheney and J. A. Spudich, Proc. Natl. Acad. Sci. U.S.A. **97**, 9482 (2000).
[4] S. Uemura, H. Higuchi, A. O. Olivares, E. M. De La Cruz and S. Ishiwata, Nat. Struct. Mol. Biol. **11**, 877 (2004).
[5] K. Svoboda, C. F. Schmidt, B. J. Schnapp and S. M. Block, Nature **365**, 721 (1993).
[6] M. L. Walker, S. A. Burgess, J. R. Sellers, F. Wang, J. A. Hammer, J. Trinick and P. J. Knight, Nature **405**, 804 (2000).
[7] Y. Oguchi, S. V. Mikhailenko, T. Ohki, A. O. Olivares, E. M. De La Cruz and S. Ishiwata, Proc. Natl. Acad. Sci. U.S.A. **105**, 7714 (2008).
[8] R. E. Cheney, Methods Enzymol. **293**, 3 (1998).
[9] E. M. De La Cruz, A. L. Wells, S. S. Rosenfeld, E. M. Ostap and H. L. Sweeney, Proc. Natl. Acad. Sci. U.S.A. **96**, 13726 (1999).
[10] H. Kondo and S. Ishiwata, J. Biochem. **79**, 159 (1976).
[11] T. Nishizaka, H. Miyata, H. Yoshikawa, S. Ishiwata and K. Kinosita, Jr., Nature **377**, 251 (1995).
[12] K. Kawaguchi and S. Ishiwata, Science **291**, 667 (2001).
[13] K. Kawaguchi, S. Uemura and S. Ishiwata, Biophys. J. **84**, 1103 (2003).
[14] S. Uemura and S. Ishiwata, Nat. Struct. Biol. **10**, 308 (2003).