Biophysical Journal, Volume 97

Supporting Material

Extensibility of Extended Tail Domain of Processive and Nonprocessive Myosin V Molecules

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SUPPELEMNETARY MATERIAL

Materials and Methods

Single-molecule force spectroscopy

Microscope coverslips (J. Melvin Freed Brand, Sigma-Aldrich) were cleaned by sonication in ethanol and followed by acetone for 20 min. Subsequently, coverslips were incubated for 20 min in concentrated H_2SO_4 and then in 6N KOH. Between each step, the slides were rinsed three times with MilliQ water. The slides were dried in a stream of clean N_2 gas. The slides were incubated for 12 h in toluene vapor containing 2% Glymo (3-glycidiloxypropyl-trimethoxisylane; Sigma-Aldrich), then washed with distilled water and dried. The Glymo-covered slides were then incubated in 10 mM Na_2CO_3 (pH 10), containing 2% (wt/vol) N-(5-amino-1-carboxypenthyl)-iminodiacetic acid (NTA; Dojindo, Tokyo, Japan) for 16 h at 65° C, washed with distillated water, and dried. For activation with Ni, the slides were incubated in assay buffer containing 10 mM $NiCl_2$ and 5 mM glycine (pH 8.0) for 2 h at room temperature. The slides were washed and stored in distilled water until further use.

The extended tail domains were diluted in buffer A to 10-100 μg/ml final concentration and allowed to bind to the tip of a gold-coated AFM cantilever (Bio-lever; Olympus, Tokyo, Japan) for 20 min.

Measurements also were performed on mica surface, which was treated the same way as the glass slides and also commercially available NTA-coated glass slides (Xenopore, Hawthorne, NJ). To explore a different approach, the His-tagged extended tail domains were immobilized to the glass surface with diazo coupling method. At the C-termini, the extended tail domains were held specifically via genetically engineered vicinal cysteine handles with a gold-covered cantilever.

To ensure that nonspecific protein-surface interaction does not compromise our measurements we employed the following experimental strategies: 1), protein concentration was kept low (10–100 μg/ml) during incubation, 2), control measurements were conducted by adding 0.2% Tween-20 in the buffer to reduce aggregation and nonspecific surface-protein interaction, 3) only data that displayed the expected contour length in repeated mechanical cycles were considered. Moreover, we performed the force spectroscopy measurements on different surfaces: NTA-coated and silanized glass and mica and ATMS coated glass (see response to second comment). In all cases, both the *Mm* and *Dm* extended tail domains behaved similarly during stretch-release experiments.

The forces versus displacement curves were corrected to obtain force versus end-to-end length. First, the zero-length, zero-force data point was obtained from the force response that corresponded to the cantilever tip reaching (or departing from) the substrate surface. The end-to-end length (z) of the tethered molecule was calculated by correcting the cantilever base displacement (s) with cantilever bending as $z = s - F/k$ (where F is the force, k is the cantilever stiffness. Forces were corrected for baseline slope obtained from the force response of the displaced but unloaded cantilever.

Protein epression, purificationand refolding of extended tail domains

The full-length mouse (*Mm)* myosin Va clone was a generous gift from Dr. John A. Hammer III (NHLBI). The extended tail domains of *Mm* myosin Va (GenBank

accession No. NM_010864 , region 2707 - 4395) and *Drosophila melanogaster* (*Dm*) myosin V (GenBank accession No. AF003826, nucleotides 2731-4200) were cloned to pET-16b expression vectors (Novagen, Darmstadt, Germany). The proteins were purified on Ni-NTA columns under denaturating conditions following the manufacturer's instructions (Qiagen, Hilden, Germany). The purified protein segments were renatured by gradual decrease of urea concentration from 8 to 0 M, using buffer A (20 mM MOPS (pH 7.2), 200 mM KCl, 5 mM DTT, 1 mM NaN_3 and 0.1 mM PMSF).

We expressed the extended tail domains of *Mm* myosin Va and *Dm* myosin V molecules. The start and the end point of fragments were chosen using Paircoil algorithm. We determined the residue-by-residue probability of amino acids lying in a coiled-coil conformation, and the N- and C-terminal ends of fragments represent the first and the last amino acid with high Paircoil value in the sequence of myosin molecules. Proteins were expressed in the BL21(DE3)pLysS system at 37° C for 3 hours in shaken cultures. We could obtain >95% pure protein preparations (Supplementary Fig. 1 *A*). The protein samples were of high purity and devoid of degradation products and did not greatly aggregate with time in solution. Samples were loaded on a gel filtration column, and they appeared as sharp, single peaks (Supplementary Fig 1. *C*). The proper dimer formation and refolding of the coiled-coil structure of the segments was confirmed using native gel electrophoresis (Supplementary Fig 1. *B*) circular dichroism (CD) spectra of the segments. On the native gel the *Dm* extended tail domain appears to be migrate faster than the *Mm* extended tail domain. This might be due to several reasons: the native gel electrophoresis was performed at low, close to *in vivo* pH (7.5), and both segments have acidic pI's (*Mm*: 5.29, *Dm*: 5.55), and also the structures of extended tail domains most likely significantly deviate from globularity.

AFM imaging

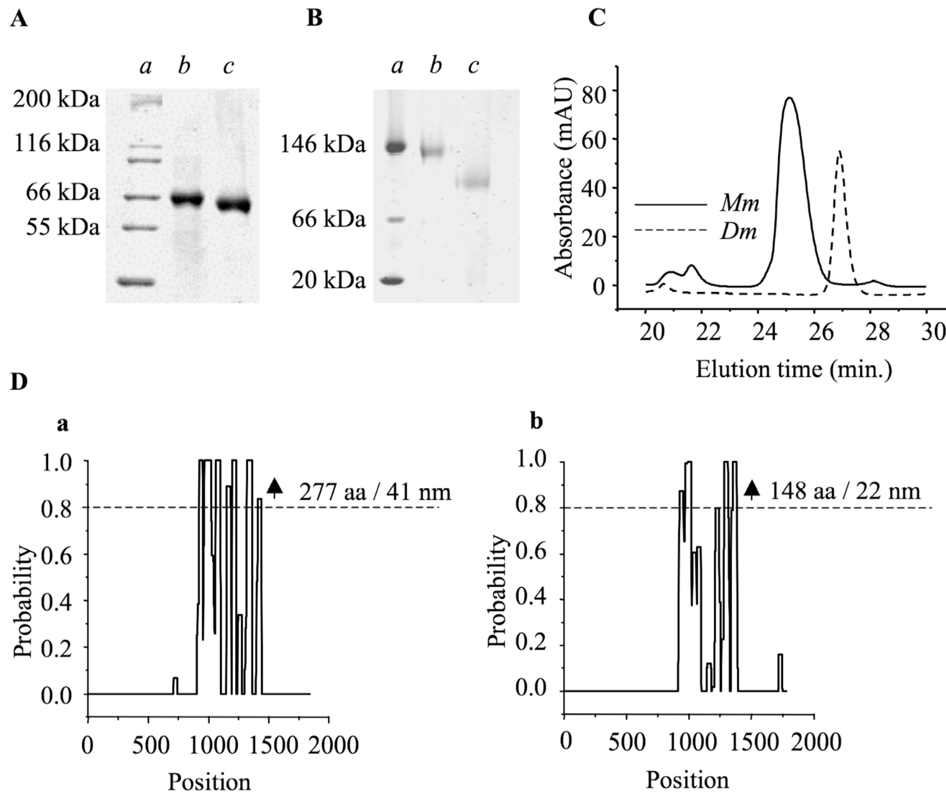
All images were acquired in tapping mode with Nanoscope IIIa controller and J scanner (Veeco, Santa Barbara, USA) under buffer A, using a short cantilever (Bio-lever; Olympus, Tokyo, Japan). The imaging was carried out at a scan rate of 0.2 Hz with a resonant frequency of ~9.2 kHz. AFM images were flattened using a first-order line fit and low-pass filtered to remove high frequency noise spikes from the images. Samples (1.5 µM final concentration) were deposited on freshly cleaved mica. Images were analyzed using Nanoscope 6.13Rsr1 software (Digital Instruments/Veeco, Santa Barbara, USA)

Electron Microscopy

Extended tail domains were diluted to 2-5 µM protein with buffer A. One drop of the sample was placed on a formvar/carbon-coated, 400-mesh copper grid previously glow discharged for 30 s in an EMScope TB500 (Emscope Laboratories, Ashford, U.K.). After 1 min, the excess liquid was absorbed with filter paper. The grid was stained with a drop of 0.5–1% aqueous uranyl acetate for 30 s, the excess stain was removed with filter paper. Micrographs were recorded at nominal magnification of 60,000x by using JEM-1200EX II transmission electron microscope (JEOL, Tokyo, Japan). The segment length was measured as the linear distance from one end to the other of the molecule using ImageJ 1.40g software (NIH, Bethesda, USA) after distance calibration.

Supplementary Figures

Supplementary Figure 1



Supplementary Figure 1. (*A*): SDS-PAGE pattern of the expressed, purified and renatured extended tail domains. Lanes (*a*): molecular weight standard *(b)*: renatured *Mm* and (c): *Dm*. (*B*): Native-PAGE pattern of renatured extended tail domains. Lanes (*a*): molecular weight standard *(b)*: renatured *Mm* and (c): *Dm* extended tail domain. (*C*): chromatogram of during gel filtration. Solid line: *Mm* extended tail domain, dashed line: *Dm* extended tail domain. (*D*): Probability of coiled-coil structure. Probabilities of forming a coiled-coil structure from 0 to 1 in the amino acid region 800 - 1500 (see **MATERIALS AND METHODS**) of *Mm* myosin Va and *Dm* myosin V (Paircoil algorithm, (*E, a*): *Mm* and (*E, b*): *Dm*).