

THE STEPS OF MUSCLE MYOSIN II

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INTRODUCTION

According to the cross-bridge theory, the chemo-mechanical cycle by which a muscle contracts is governed by a deterministic process that stipulates the myosin molecules are only able to pull the actin filaments, or perform the power stroke, in a specific direction (Huxley, 1957). Each myosin has a mid axis symmetry such that half myosins pull the actin filaments toward the middle of the sarcomere, thereby causing sarcomeres to shorten. However, in experiments of single actin-cross-bridge interactions, it has been shown that cross-bridges pull actin filaments in both directions: a preferred direction towards the positive charged end of actin (forward steps), and a less frequently observed stepping towards the negative end of actin (backward steps). However, it is not clear if the direction of cross-bridge stepping is determined by the polarity of actin or the orientation of the cross-bridge. The purposes of this study were (i) to determine if the preferred direction of pulling is governed by the orientation of the myosin cross-bridge or the polarity of the actin filament, and (ii) to quantify if there are mechanical differences between forward and backward steps.

METHODS AND PROCEDURES

A three-bead laser trap setup, as first developed by Finer et al. (1994), was used for testing single cross-bridge interactions with actin (Figure 1). Briefly, two micron-sized beads were attached to the ends of the actin filament and were held by two independently

controlled lasers for manipulation, force and step size measurement. A cross-bridge (a heavy meromyosin, double headed construct) was placed on top of a surface fixed bead for interaction with the actin filament. Upon attachment of the cross-bridge, the direction of actin movement, force, step size, and the time of attachment (dwell time) were recorded at low ATP concentrations, so that attachment events could be clearly identified. Once a set of cross-bridge attachment events had been recorded for a given construct, the actin filament orientation was changed by 180° so that the plus and minus end of the filament were reversed and a second set of attachment events were recorded in this new configuration.

Non-parametric tests were used to determine if there was a significant difference between dwell times, step sizes and forces of the forward and backward steps

RESULTS

Step size and associated force were greater for forward ($15 \pm 1\text{nm}$) than backward steps ($12 \pm 1\text{nm}$), while dwell times were the same. Backward steps (32%) occurred in random order.

Following the change in actin orientation by 180°, the primary stepping direction was reversed (Figure 2).

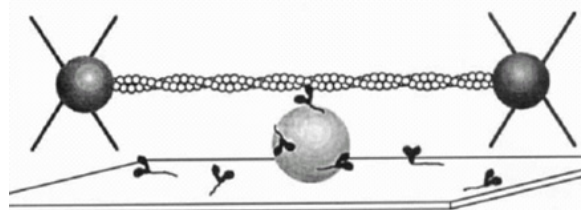


Figure 1. “Dumbbell” setup first proposed by Finer et al. (1994). Two micron sized beads hold an actin filament in place as it interacts with a single cross-bridge.

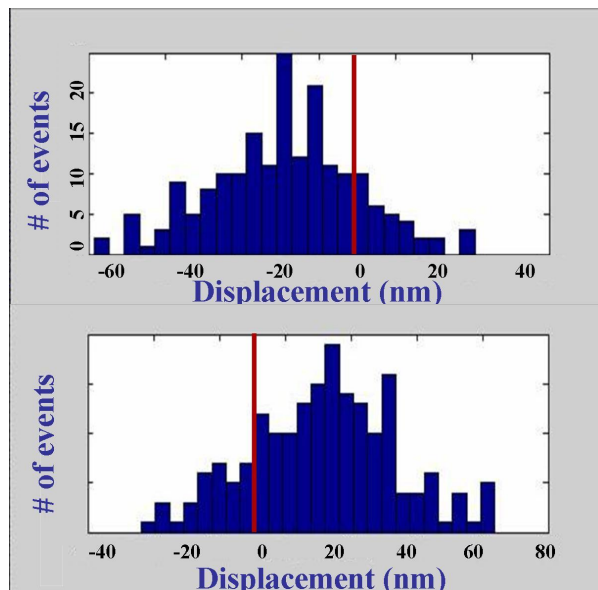


Figure 2. Displacement histogram showing the number of forward and backward steps before and after a 180° change in direction of an actin filament. Note the change in preferred step direction following actin reorientation.

DISCUSSION

Single cross-bridge interactions with actin show a preferred direction that depends on the orientation of the actin filament. Thus, we conclude that it is likely actin orientation, and not cross-bridge orientation, that determines the direction of force production in sarcomeres. However, there were a significant number of “backward” steps, indicating that cross-bridge interactions with actin are not deterministic as described in traditional cross-bridge models (Huxley, 1957; Huxley and Simmons, 1971).

Step size and force were significantly greater for “forward” compared to “backward” steps, suggesting that whatever the detailed molecular mechanisms of actin-myosin

interaction, they are more effective in the more frequently occurring forward direction. Backward steps have been observed previously in laser trap setups (Kitamura et al, 2005, Guilford et al., 1997, Veigel et al., 1998), but to a smaller degree. Possibly, the relatively soft laser trap setup used here, which allows for large Brownian motion of the actin filament, might partly be responsible for the high percentage of backward stepping and it would be interesting to determine if trap stiffness, and by implication the amount of Brownian motion, affects the percentage of observed backward steps.

Dwell times were the same for the forward and backward steps, indicating that the basic ATP hydrolysis kinetics of the cross-bridge cycle was the same for both step directions.

SUMMARY

The results of this study indicate that the polarity of the actin filament determines the direction of force application and that actin-myosin interactions, at least in a three bead laser trap setup, are not deterministic as described in traditional cross-bridge models.

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