INTRODUCTION

Increases in sarcomere length can reduce thick-to-thin filament spacing in skinned muscle fibers, thereby increasing force production at longer sarcomere lengths [1,2]. However, it is unclear how changes in sarcomere length and lattice spacing affect cross-bridge kinetics at fundamental steps of the cross-bridge cycle, such as the MgADP release rate. Changes in this step can significantly alter cross-bridge behavior, as the MgADP dissociation rate is the primary determinant of myosin attachment duration under saturating MgATP conditions [3,4]. We hypothesize that decreased lattice spacing, achieved through increased sarcomere length or osmotic compression of the fiber via dextran T-500, could slow MgADP release rate and increase cross-bridge attachment duration. To test this, we measured cross-bridge cycling and MgADP release rates in skinned soleus fibers using stochastic length-perturbation analysis at 2.5 and 2.0 µm sarcomere lengths as [Ca2+] and [MgATP] varied.

METHODS

To investigate the effect of sarcomere length and thick-to-thin filament spacing at specific steps of the cross-bridge cycle, we used stochastic length-perturbation analysis to measure myosin kinetics at 2.0 or 2.5 µm sarcomere length in skinned rat soleus fibers, in the presence or absence of 4% dextran T-500. This length-perturbation analysis allows us to measure the stress-strain response of contracting muscle fibers over a broad frequency-range using a short burst of band-limited Gaussian noise. As sarcomere length varies, frequency-dependent shifts in this stress-strain response result from varied cross-bridge kinetics at specific steps in the cross-bridge cycle. These include MgADP release and MgATP binding rates, which we measured by titrating the [MgATP] of activating solution.

RESULTS AND DISCUSSION

Myosin detachment and MgADP release rates were slower at 2.5 µm vs 2.0 µm sarcomere length at both submaximal and saturating [Ca2+] in the absence of dextran (Figure 1). Myosin detachment and MgADP release rates were also slower at 2.5 µm vs 2.0 µm sarcomere length when fibers were compressed with 4% dextran T-500. However, we did not observe significant differences in these rates between fibers at the same sarcomere length with and without dextran. These data suggest that detachment of myosin from actin is slowed as sarcomere length increases due to slower cross-bridge MgADP release rate, but that this process may not be tied exclusively to changes in myofilament lattice spacing. At a longer sarcomere length, the greater forces borne by the myofilaments diminish their relative extensibility, thereby increasing the load that myosin experiences throughout a cross-bridge cycle. This enhanced load or molecular strain on the motor domain may slow the series of conformational changes required for MgADP release from the nucleotide binding pocket and slow cross-bridge detachment rates. This mechanism may serve to enhance the efficiency of MgATP utilization at longer sarcomere length by prolonging the time myosin spends in the strongly-bound, force generating phase of the cross-bridge cycle.

CONCLUSIONS

The rate of MgADP release is dependent on sarcomere length, but is not consistent with changes in lattice spacing. These data suggest that skeletal muscle exhibits sarcomere-length dependent changes in cross-bridge kinetics and MgADP release that are separate from, or complementary to, changes in lattice spacing.

REFERENCES


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