How Actin-Myosin Interactions Differ With Different Isoforms of Myosin

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The development of in vitro techniques for assaying the mechanical properties of individual actin-myosin interactions has provided investigators with a powerful tool to address questions about fundamental properties of the force-generating reactions that produce movement of cells or organelles within cells. These techniques are capable of measuring the force and/or displacement produced by the interaction of a single force generator with a single actin filament. The load on the force generator can be varied in a controlled manner to allow the sampling of force and velocity under a variety of conditions. Progress is being made toward measuring the amount of ATP split during these reactions. There are, however, limitations to the existing in vitro motility assays, such as the inability to control the orientation of the molecules rigorously and the quantitative effects of brownian motion on the force and displacement transients that are produced by the interaction between myosin and actin. One consequence of these problems and the different approaches of the several laboratories using in vitro assays is the existence of a range of values for the force and displacement produced by the unitary force-generating set of reactions. Most results indicate forces between 2 and 10 pN and displacements from 5 to 15 nm with means of about 5 pN and 5 nm, respectively. The latter is about the periodicity of actin in the thin filament. In spite of these problems, which are slowly responding to the ingenuity of investigators using the techniques, much useful information has been produced. (For a more detailed discussion of the application of in vitro motility techniques to the study of the mechanics of the interaction of actin with myosin, see References 1 and 2.)

In intact cardiac tissue and cells, unloaded shortening velocity in muscles homogeneous for β-myosin heavy chain (V 1) is less than 40% of that in muscles homogeneous for α-myosin heavy chain (V 3). The rate of hydrolysis of ATP by myosin is similarly slower when the heavy chain is the beta isoform. The mechanical basis for these differences has not been shown. In addition, there has been controversy about whether the individual V 1 force generators produce more force per cycle and greater time-averaged force than V 3 ones. An understanding of the mechanism for the different rates of shortening and ATP hydrolysis has broader implications than just details of the function of V 1 and V 3. Comprehension of the basis for this difference should lead to further insights into the mechanism and reactions that produce force.

In an important application of the in vitro motility assay to the study of cardiac muscle, Sugiura et al. have addressed the question of the basis for the differences in the unloaded shortening velocities and ATPase activities in cardiac muscle containing α- or β-myosin heavy chain. In their in vitro motility assay, a single myosin molecule interacts with an individual actin filament attached to a bead, which in turn is suspended by “optical tweezers” that can control the stiffness of the preparation. With stiffness set at a high level, the bead is held stable, and isometric contractile force can be measured. When the stiffness is low, displacement of the actin filament occurs, as in a lightly loaded isotonic contraction.

Sugiura et al. found that V 1 and V 3 force generators produce the same force and the same displacement. The difference between the force and displacement transients of the two is in the duration of the individual events. Both the force and the displacement transients are approximately 40% longer with V 3 than with V 1. These results provide the strongest evidence to date in favor of a difference in duration without a difference in generation of force.

The results of Sugiura et al. lead to some new and interesting questions. Although there is remarkable homology in amino acid sequence between α- and β-myosin heavy chains, there are differences at several crucial locations in the molecule, including the rod, the hinge, the light chain domain, the actin binding site, and the ATP binding site. The difference in the durations of the force and displacement transients of V 1 and V 3—about 40% —is significantly smaller than the 150% difference in unloaded shortening velocity found in intact cardiac cells and in velocity measured when multiple actomyosin interactions occur simultaneously. The experiments should detect functional differences due to amino acid differences in the myosin head but probably not in the rod. The discrepancy in the size of the effect of isoform on velocity of shortening could be due to difference in the conditions under which myosin and actin interact in the in vitro assay or the existence of another factor contributing to the slower cycling of V 1 myosin. For instance, a difference in lateral interactions between rod portions of myosin in the intact filament could alter compliance in thick filaments and modify force and displacement.

The velocity of shortening for V 1 that was measured in the in vitro assay, approximately 45 nm/s, is considerably slower by more than an order of magnitude than that measured in intact sarcomeres, but this can be attributed to the low concentration of ATP used in the in vitro assay. In order to be able to resolve individual contractile events, 0.5 µmol/L ATP was used. At this concentration of ATP, there is a marked
limitation in the velocity of shortening in intact cardiac cells and in the rate of ATP hydrolysis in actomyosin in solution.

An additional provocative result obtained by Sugiura et al.\(^5\) is the difference in the duration of the force and displacement transients regardless of the isoform of myosin heavy chain. The force transient is about 65% longer than the displacement transient with each isoform. This suggests that the rate of detachment of myosin from actin, which is consistent with first-order kinetics in their data, is either dependent on strain or inversely related to load. Intuitively, the latter seems less likely than the former. Huxley\(^7\) first proposed a relation between strain and detachment rate in his presentation of a model for crossbridge cycling, and evidence to support it has been generated subsequently. If it is feasible to analyze the shape of the transient, either by achieving greater time resolution, time-averaging multiple transients, or using various analogues of ATP, it should be possible to learn more about the factors that influence the rate of detachment of myosin from actin and gain additional information about the mechanical correlates of different chemical events within the crossbridge cycle. This is an exciting prospect.

With this study, Sugiura et al.\(^5\) have provided answers to some questions about the force-generating reactions between actin and myosin and posed others that have broad implications for all molecular motors.

References


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