Comment to the Editor

Response to Bianco et al.: Interaction Forces between F-actin and Titin PEVK Domain Measured with Optical Tweezers

ABSTRACT A recent publication in *Biophysical Journal* by Bianco et al. ("Interaction forces between F-actin and titin PEVK domain measured with optical tweezers") shows that the PEVK domain of titin molecules interacts with F-actin. This newly discovered behavior could influence the mechanical properties of striated muscles, and Bianco et al. suggest that the interactions between actin and titin could modulate thixotropic behavior. In this Comment to the Editor, we suggest that the thixotropic properties of striated muscles in vivo are more likely to reflect dynamic changes in the proportion of myosin cross-bridges bound between the myofilaments.

Thixotropy is the term used in the biomedical literature to describe the temporary reduction in stiffness that a muscle exhibits following an imposed movement (1,2). The behavior was demonstrated in relaxed cat semitendinosus muscle by Denny-Brown (3) but the first authors to apply the specific term ''thixotropy'' to muscle were Buchthal and Kaiser (4). A recent publication by Bianco et al. (5) suggests that thixtropic behavior may reflect the strain dependence of relatively long-lasting interactions between actin filaments and the PEVK region of titin molecules.

Measurements using intact skeletal muscle fibers (6) show that thixotropic behavior in relaxed muscles is primarily due to the history dependence of the short-range elastic component (7). Specifically, the initial stiffness of a relaxed intact muscle is reduced during the second of two length changes if the movements are imposed closely one after the other. Herbst (8) postulated that the short-range elastic component represents the mechanical effects of a small population of slowly cycling cross-bridges (an idea originally proposed by D. K. Hill (7)) and that thixotropic behavior in relaxed muscles reflects a temporary reduction in the number of attached cross-bridges following a mechanical perturbation. We published experiments and simulations that support this hypothesis in 1998 (9) but the theory remains controversial (10).

Bianco et al.'s new findings (5) increase the probability that thixotropic behavior in relaxed muscle reflects a non-cross-bridge mechanism. Nonspecific titin PEVK-actin interactions could produce a short-range tension response and it seems likely that the number of bonds linking the actin and titin filaments in a relaxed muscle would increase with time if sarcomere length were held constant. These properties are probably sufficient to explain the basic features of thixotropic behavior in relaxed muscles.

The drawback of proposing titin PEVK-actin interactions as a general mechanism underlying muscle thixotropy is that the theory does not readily explain the history-dependent behavior that we now know to be a property of contracting muscle as well. Experiments using chemically permeabilized skeletal muscle fibers (11,12) activated in solutions with different free Ca^{2+} concentrations show that prior movement temporarily reduces the stiffness of contracting fibers and that the size of the thixotropic reduction in stiffness scales with the free Ca^{2+} concentration in the same way as active force. Additional measurements using isolated myocardial preparations (13) show that 2,3 butanedione 2-monoxime, a chemical inhibitor of myosin force development, eliminates the short-range force response and dramatically reduces the history dependence of the observed mechanical properties. These observations provide strong support for Herbst's original hypothesis (8) that muscle thixotropy primarily reflects temporary changes in the number of attached cross-bridges.

Titin PEVK-actin interactions are not, in our opinion, the most likely explanation for the general property of muscle thixotropy but Bianco et al.'s recent observations are important and significant. The new finding that titin PEVK domains interact with actin filaments could explain why force-length curves measured during lengthening and shortening movements are slightly different for permeabilized muscles immersed in solutions with very low (nanomolar) free Ca^{2+} concentrations (12). The long-lived nature of the titin PEVK-actin bonds means that they could also explain the small biphasic tension responses that are observed in relaxed frog muscles subjected to ultraslow ($<1 \times 10^{-5}$ muscle lengths s^{-1}) length changes (9).

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