left out the reaction scheme, the authors cannot reasonably use the scheme to test for the validity of force generation localized to actin-bound cross-bridges by varying [P_i].

On the other hand, if the more traditional reaction in Eq. 3 replaces the concerted reaction in Eq. 1 as the reaction that is taken to be near equilibrium, then the change in force observed by Baker and his colleagues is tightly coupled to the action of P_i localized to individual cross-bridges. The decrease in force with increasing [P_i] is then due to P_i binding to A.M.D. This increases [A.M.D.P_i], which is not generating force, at the expense of [A.M.D], which is. This redistribution of force-producing cross-bridge states does not require a redistribution of cross-bridge orientations. The binding of P_i also reduces the affinity of a cross-bridge for actin, which will reduce the number bound. Cross-bridges dissociated by ATP binding to A.M certainly change orientation as they go through the hydrolytic cycle. To what degree the P_i-induced increase in [A.M.D.P], which is in rapid equilibrium with an increased [M.D.P], will contribute to changes in orientation is not as clear. What is important is that when the intermediate A.M.D.P is included in the reaction, force production remains localized at the individual cross-bridges and dependent on [P_i], in which case the reported measurements actually confirm the assumption that force and chemistry are localized to the actin-bound cross-bridge.

Supported by National Institutes of Health grant AR42895.

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Muscle Chemistry and Force

In a recent article (Baker et al., 1999), we reported that the distribution of myosin orientational and biochemical states is independent of P_i-induced changes in the force of fully activated isometric muscle, despite observations that this distribution does vary with calcium-induced changes in the force of partially activated isometric muscle (Ostap et al., 1995; Baker et al., 1998; Brust-Mascher et al., 1999). We then showed that a simple chemical thermodynamic analysis directly explains these data and challenges the independent force generator model of muscle contraction. In his Letter to the Editor of the *Biophysical Journal*, Highsmith (2000) presents an accurate summary of our article followed by an intriguing and testable alternative interpretation of our data. Specifically, Highsmith proposes that the independent force generator model might still be consistent with our data, if changes in muscle force were localized to internal conformational/biochemical changes of actin-attached myosin cross-bridges and if global rotations of myosin cross-bridges only occurred when muscle is allowed to shorten. Highsmith does not specify the nature of the internal myosin conformational change that he believes is responsible for a P_i-induced decline in muscle force. What our data require (Baker et al., 1999) is that Highsmith's proposed conformational change is not detected in our electron paramagnetic resonance (EPR) studies and is distinct from the myosin conformational changes correlated with force generation upon muscle activation, i.e., disorder-to-order in the myosin catalytic domain and a distinct rotation of the myosin light-chain domain (Ostap et al., 1995; Baker et al., 1998; Brust-Mascher et al., 1999). Although the mechanism proposed by Highsmith is feasible, interesting, and important to consider, it has not been incorporated into the independent force generator formalism (Huxley, 1957; T. L. Hill, 1974) and tested against our data; thus, it does not constitute

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Received for publication 27 March 2000 and in final form 26 May 2000.

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0006-3495/00/09/1687/02 $2.00

Biophysical Journal 79(3) 1688–1689

an alternative to our model, nor does it formally refute our conclusions.

Highsmith further suggests that the independent force generator model might be able to explain our data if the ternary complex, A.M.D.P_i, were explicitly included in our reaction scheme. Specifically, Highsmith suggests that an increase in [P_i] would shift the distribution of myosin heads from A.M.D to A.M.D.P_i, and this shift might not be detected in our experiments. However, if the M.D.P_i to A.M.D.P_i transition is reversible, mass action would further shift myosin heads from the A.M.D.P_i to the M.D.P_i state, and this shift would be detected in our experiments (Ostap et al., 1995). Much work supports the hypothesis that transitions among all weak-binding states are reversible (Eisenberg and Hill, 1985), and in the model proposed by Highsmith, M.D.P_i and A.M.D.P_i are both weak-binding (non-force-producing) states. If the transitions among the M.D.P_i, A.M.D.P_i, and A.M.D states are near equilibrium, the chemical potential of the A.M.D.P_i state cancels out of the free energy equation (Eq. 2 in Baker et al., 1999), and Eq. 2 applies regardless of whether the A.M.D.P_i state is explicitly included in the reaction scheme.

EPR of spin-labeled myosin in muscle has revealed an unexpected correlation between active, isometric muscle force and myosin biochemistry (Baker et al., 1999). We have modeled these data by applying basic chemical principles to a well-defined rotating cross-bridge mechanism (Baker et al., 1999), and we have shown that this formal model accurately describes steady-state muscle mechanics, energetics, and biochemistry (Baker, 1999). Until Highsmith's proposal can be developed into an equally self-consistent independent force generator model, we maintain that our data challenge the assumption of independent force generators in muscle. Though this conclusion may be controversial, it is certainly not new; others before us (Leibler and Huse, 1993; Jülicher and Prost, 1995; Vilfan et al., 1998) have suggested that cooperative interactions among cross-bridges in muscle might be considered. Moreover, our model is consistent with the classic muscle model of A. V. Hill (1938) in which he described mechanochemical coupling at the level of the macroscopic muscle system, not at the level of the individual molecules in that system. As discussed in our paper, our data imply that the chemical energy available for work by muscle, RTln([A.M.D]/[M.D.P_i]), is proportional to the macroscopic muscle force (Eq. 4 in Baker et al., 1999). In fact, we have shown (Baker, 1999) that A. V. Hill's energy equation and force-velocity relationship (A. V. Hill, 1938) can be directly obtained from Eq. 4 in Baker et al. (1999). In essence, a simple chemical thermodynamic analysis of our EPR data provides a molecular basis for A. V. Hill's model.

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