Pollard to Actomyosin: "Freeze! Don't Even Move Your Head"

David D. Thomas
Department of Biochemistry, University of Minnesota Medical School, Minneapolis Minnesota 55455 USA

In the field of muscle biophysics, nothing has attracted more attention (or generated more controversy) than the Big Question: Does myosin rotate on actin to generate force? For at least two decades, textbooks have shown attractive and compelling cartoons in which the myosin head forms a *weak*-binding cross-bridge to actin at a 90° angle, then becomes a *strong*-binding cross-bridge at a 45° angle, thus pulling the actin filament by 5–10 nm and generating force. However, compelling evidence for this two-angle rotating cross-bridge model has not been produced, either because *(a)* the model is not correct or *(b)* the force-generating actomyosin ATPase cycle is so dynamic that direct structural evidence is very difficult to obtain. The strong-binding 45° structure at the end of the ATPase cycle (AM.ADP or AM) has been observed clearly and directly by electron microscopy, since it can be obtained at equilibrium. The missing link is a convincing observation of the more elusive weak-binding structure that occurs earlier in the cycle (AM.ATP or AM.ADP.P).

A report by Pollard et al. (1), in this issue, represents an impressive effort to test this hypothesis by direct electron microscopic visualization of the actomyosin complex during the active interaction of myosin, actin, and ATP. They chose to investigate this problem in a solution containing isolated myosin heads (S-1) and actin, because detailed kinetic studies of this system have shown clearly that the desired weak-binding cross-bridges predominate in the steady-state of the ATPase cycle. They used a novel stopped-flow/rapid-freezing apparatus to trap these steady-state intermediates, then etched and replicated the samples with platinum and carbon for examination by electron microscopy. The distribution of myosin head angles relative to the actin filaments was analyzed quantitatively, using procedures designed to eliminate bias. A remarkable result was obtained: *no effect of ATP.* Whether the samples were frozen in the absence of ATP (the rigor complex, AM), in the steady-state of ATP hydrolysis (predominantly the weak-binding states AM.ATP and AM.ADP.P), or after all the ATP had been hydrolyzed to ADP (AM.ADP), most of the actin-attached myosin heads were found to be oriented between 30° and 50°, with a mean angle ~ 40°. Therefore, these results appear to exclude any model in which states occurring early and late in the "power stroke" have substantially different angles (e.g., 45° different).

This conclusion is controversial not only because it is inconsistent with the textbook cartoons, but also because it appears to contradict other recent EM results that showed a broad distribution of angles for myosin heads attached to actin in the presence of ATP (2, 3). However, in those studies, the myosin heads (S1) were covalently cross-linked to actin. Pollard et al. (1) report that their rapid-freeze method confirms this ATP-induced disorder only when S1 is *cross-linked* to actin, and suggest that the cross-linking gives misleading results due to the disorder of S1 molecules that are actually detached from their binding sites on actin but are tethered by the covalent cross-links. This explanation can not be applied to the results of Katayama (4), who used a similar quick-freeze/deep-etch technique and reported significant changes in the shapes of attached S1 in the presence of ATP. However, key differences in Katayama's experimental conditions, resulting in a much lower fraction of bound S1 and possible artifacts due to immobilization of the actin on mica, cast doubt on the significance of those observations.

The technical limitations and low resolution of this stopped-flow/rapid-freeze EM method, combined with the dynamic nature of the actomyosin system, make it difficult to rule out artifacts. Are the actin-bound heads seen in the EM representative of the ones present just before freezing? Unfortunately, Pollard et al. (1) were not able to quantitate the fraction of attached heads during the steady state and thus to verify that this fraction agreed with that predicted by biochemical measurements. It remains possible that the freezing and etching and replication does not preserve the attachment, or at least the angle of attachment, of the weakly attached heads, which are known to undergo association and dissociation on the sub-millisecond time scale.

EPR spectroscopy is complementary to EM, since it can be performed in solution without fixation or replication, and it can quantitate and resolve heads that are in different orientational or rotational states. EPR on solutions of S1 and actin shows that a spin label on the myosin head is rigidly immobilized in the absence of ATP but undergoes substantial microsecond rotational motion (through at least 45°) when the head is attached to actin in the steady-state of the ATPase cycle (5). Analogous experiments in relaxed muscle fibers at low ionic strength showed that most spin-labeled heads are dynamically disordered, with an angular width of at least 45°, under conditions where the heads have mechanical properties consistent with weak (rapid equilibrium) attachment (6). These EPR results seem consistent with the ATP-induced disorder concluded from other EM studies

0006-3495/93/02/297/02 $2.00

(2, 3). However, one key aspect of the EPR results in muscle fibers agrees with the conclusions of Pollard et al.: the small fraction of heads that *are* well oriented have a rigor-like angle, whether in low ionic strength relaxed muscle (6) or isometrically contracting muscle (7). Thus, the key conclusion of Pollard et al. (1) remains consistent with most spectroscopic and EM data: *no well defined cross-bridge angles are observed that are significantly different from the rigor (AM) angle at the end of the power stroke (8).*

The results of Pollard et al. (1) add to the evidence that the myosin head does not rotate substantially on actin during force generation (8). Among the many alternatives, including some in which the myosin head undergoes no large rotations or structural changes, Pollard et al. favor the proposal that most of the head's mass (including most spectroscopic probes) remains fixed on actin, but that a structural change within the head causes a substantial rotation of the distal part of the head, which might not be detected by this relatively low-resolution EM technique. This hypothesis is supported by recent x-ray scattering measurements that suggest a substantial structural change in S1 due to ATP hydrolysis (9). The testing of this and other hypotheses will require further structural and spectroscopic studies that focus on the distal region of the head, both in solution and in active muscle fibers. Another important goal is to obtain structural and spectroscopic data in the transient phase of the ATPase cycle, to be sure that short-lived intermediates are not missed.

Received for publication and in final form 14 October 1992.

REFERENCES

1. Pollard, T. D., D. Bhandari, P. Maupin, D. Wachsstock, A. G. Weeds, and H. Zot. 1993. Direct visualization by electronmicroscopy of the weakly bound intermediates in the actomyosin ATPase cycle. *Biophys. J.* 64:454–471.
2. Craig, R., L. E. Greene, and E. Eisenberg. 1985. Structure of the actomyosin complex in the presence of ATP. *Proc. Natl. Acad. Sci. USA.* 82:3247–3251.
3. Applegate, D., and P. Flicker. 1987. New states of actomyosin. *J. Biol. Chem.* 262:6856–6863.
4. Katayama, E. 1989. The effects of various nucleotides on the structure of actin-attached myosin subfragment-1 studied by quick-freeze deep-etch electron microscopy. *J. Biochem.* 106:751–770.
5. Berger, C. L., E. C. Svensson, and D. D. Thomas. 1989. Photolysis of caged ATP induces microsecond rotation of myosin heads on actin. *Proc. Natl. Acad. Sci. USA* 86:8753–8757.
6. Fajer, P. G., E. A. Fajer, M. Schoenberg, and D. D. Thomas. 1991. Orientational disorder and motion of weakly attached cross-bridges. *Biophys. J.* 60:642–649.
7. Cooke, R., M. Crowder, and D. D. Thomas. 1982. Orientation of spin-labels attached to cross-bridges in contracting muscle fibers. *Nature* (*Lond.*) 300:776–778.
8. Thomas, D. D. 1987. Spectroscopic probes of muscle crossbridge rotation. *Annu. Rev. Physiol.* 49:891–909.
9. Wakabayashi, K., M. Tokunaga, I. Kohno, Y. Sugimoto, T. Hamanaka, Y. Takezawa, T. Wakabayashi, and Y. Amemiya. 1992. Small-angle synchrotron x-ray scattering reveals distinct shape changes of the myosin head during hydrolysis of ATP. *Science* (*Wash. DC*). 258:443–447.