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A GUIDE TO THE MUSCLE PAPERS

Manuel F. Morales

Cardiovascular Research Institute, University of California, San Francisco

INTRODUCTION

In preparing this introduction I am in the advantageous position of heralding what I have already heard – a fact that largely accounts for any insight that may follow. It was clear at this Conference, as it is from the contemporary literature, that a major problem in muscle is on its way to solution, while another is just taking form. I will structure the introduction accordingly, after explaining a little more carefully what I mean.

Accepting what is no longer debatable – that muscle "runs on" ATP hydrolysis and that its multiple force generators cause filaments to slide past one another, we have (Problem I) to figure out the work cycle of an individual force generator (myosin cross-bridge and actin bearing), including the correlation between positions of the moving parts and stages in fuel degradation. This figuring out is going on right now, with considerable success, even though nothing like unanimity or conviction has yet emerged. Inside Problem I there is a deeper problem (Problem II). The moving parts (probably the S2 and S1 moieties of the myosin molecule) are proteins, with complex atomic topographies, and these topographies catalyze transitions, or control transition rates, or generate torques. Understanding these "local conformational changes" is in its infancy, yet important progress is being made.

SECTION I

Our present understanding of Problem I results from many, and multiply authored, ideas; space limitation (quite unfairly) allows me to credit only the more recent ideas, and to indicate in brackets, [ ], how the Conference relates to them. Of the three moieties of a half-myosin molecule, "LMM" is rigidly held in the thick filament structure, "S2" is a long, slender middle piece attached to LMM by a hinge of unknown properties [D. L. Taylor describes a novel way of detecting S2 movement in the organized fiber], and "S1" is an ellipsoid, roughly four times as long as it is wide. Time-resolved fluorescence polarization decay suggested that S1 attaches to S2 by a frictionless swivel (1) [J. C. Seidel describes a new EPR method that assures this conclusion and shows great promise for the detection of "slow" molecular rotations]. Existence of this swivel provides a molecular understanding of how, in organized fibers, S1 can be either perpendicular to the fiber axis (in "relaxation") or angled to it (in "rigor") – a crucial observation first made by Reedy et al. (2) [M. K. Reedy documents this observation].

Properly defined measurements of (S1)-to-actin affinity are only now being made, but there are many indications that "under physiological conditions" of temperature, solvent, etc., it is a very high affinity, as is the (S1)-to-ATP affinity. It is also well known that these affinities are competitive, but mediated by distinct binding sites (3). [A. Martonosi presents data which, under one interpretation, permit the calculation of the interaction free energy between sites.]

Knowing the Michaelis constant (4) of myosin ATPase, and the overall free energy of ATP hydrolysis (5), we could long ago suggest (6) that "coupling," or the transfer of free energy between subsystems, occurs in the binding process, leaving a rather small ΔG to be associated with the hydrolysis on the enzyme surface. Although this suggestion seems essentially correct, it has been assured only by modern work, which in turn had to await the kinetic analysis of ATPase. That in the sequence of myosin ATPase intermediates there should be an M·ATP is obvious, and M·ADP was reasoned as well as demonstrated two decades ago (7). More subtle methods were needed before an "in-between" species – a "special" complex of myosin and hydrolytic products – could be found. One path of discovery turned out to be the study of isotopic exchanges – the necessity of inventing a species to account for multiple ^{18}O exchange between H_2O and the P_i product [P. D. Boyer and R. G. Yount discuss their respective pioneering findings], and also the necessity of inventing a "special" M-ADP complex to account for ^{32}P exchange between the P_i product and the parent ATP [K. Hotta discusses his findings]. The other path of discovery was the use of "reporter" probes. Morita (8) was first in the use of tryptophane absorbance to detect nucleotide binding, and the first detection of a conformational alteration coexistent with steady-state ATPase was made by Cheung (9). However, the realization that the "special" probe signal issues from the intermediate of maximum concentration, i.e. the intermediate preceding the rate-limiting step, was first expressed by Seidel and Gergely [J. Gergely discussed this conclusion using a spin label attached to S1, and later (but independently) by Werber, Fasman, and Szent-Gyorgyi [A. Szent-Gyorgyi discusses this work] using intrinsic tryptophane fluorescence. The latter observations, especially, opened the way for a clever and thorough kinetic analysis of myosin ATPase by D. R. Trentham and his associates at Bristol [C. Bagshaw summarizes this work]. The analysis identifies the species of maximum concentration as a "special" M·ADP·P_i, preceding the rate-limiting partial reaction. A by-product of the analysis is that the surface hydrolysis preceding this species is accompanied by a small $-\Delta G$; Wolcott and Boyer reached the same conclusion, and pointed out that the $-\Delta G$ was particularly small relative to the $-\Delta G$ of ATP-to-(S1) binding, thus bearing out our old prophecy.

Since under "physiological conditions" actin powerfully activates myosin ATPase, it is obvious that kinetic analysis must be extended to actomyosin ATPase. But simple reasoning suggests that (accepting Lymn and Taylor's (10) deduction of very quick actomyosin dissociation by ATP) in the sequence beginning with M·ATP, the first myosin species to combine with actin must be the "special" complex, M·ADP·P_i (11). Such evidence as exists for this complexation, however, indicates that it is made with modest affinity [K. Hotta reports on the influence of actin and of "negative work" on the special complex]. We have suggested (12), for example, that the special complex can be simulated by M-S-S-DP (here DP stands for thio-IDP). Stone (13) has found that the

spin signal from this analog does indeed resemble that from the special complex. Actin increases the viscosity of myosin so labeled, according to Stone, but only to a small extent, indicating that only a small $-\Delta G$ is associated with the attachment of the complex to actin. By contrast, the attachment of nonligated S1 to actin (to form the "rigor complex") entails a very large $-\Delta G$. If the special complex attaches to actin with a weak affinity and then, while attached, passes over into the rigor complex, the transition that occurs while attached must entail a large $-\Delta G$.

The foregoing ideas about the attachment of the special complex to actin, and the subsequent exergonic transition, harmonize in a most satisfying way with results of mechanical and structural investigations. H. E. Huxley (14) discussed two quite opposite views of how a crossbridge might work. In one there need be no hinges or swivels, but the S1, being highly deformable, can "massage" the actin by; in the other the S1 is rigid, but the (S1)–(S2) hinge allows the distal end of S1 to roll on actin, thus passing from an attitude in which the major axis of S1 is perpendicular to the fiber axis to one in which it is angles to the axis. The absence of any ATP-induced change in rotational mobility [R. A. Mendelson et al. discuss fluorescence depolarization evidence] or global CD spectrum [J. Y. Cassim discusses CD evidence] argues strongly against any highly deformable S1. On the other hand, A. F. Huxley and R. L. Simmons have strongly supported and extended the opposite model with observations on tension transients following sudden changes in length [A. F. Huxley discusses these results]. To correlate chemical ideas with the Huxley-Simmons view, one need only assume that S1 in the special complex attaches with its major axis perpendicular to the fiber axis, and that it then, by proximally rotating about its swivel and distally rolling on actin its major axis, becomes angled to the fiber axis. Using polarization of the tryptophane fluorescence from fibers as a phenomenological indicator of S1 attitude, dos Remedios et al. (12) found results of just this kind, since the attitude of S1 in the M-S-S-DP form was like that of S1 in relaxation. In the Huxley-Simmons view it must be assumed that the turning torque on S1 arises at the (S1)-actin interface, not at the swivel. This feature of the model has been supported by a neat experiment of Nihei et al. (15), who found that when "contraction" or "rigor" solutions were presented to S1 initially in "relation" no rotation occurs unless actin is opposite to the S1.

All the considerations of this section can be put together in a diagram (Fig. 1) of the working cycle executed by a single crossbridge and bearing. I believe that cycles like this are in the minds of many contemporary students of contractility. I have tried to suggest some energetic correlates of the cycle. While this cannot be done rigorously, it is nonetheless useful to think how parts of the complete system interact with one another during the "one-way" degradation of G pictured on the right hand side of the diagram. In particular, note during the initial step that two transactions each involving large $[\Delta G]$ oppose one another so that the system as a whole probably loses little in G, saving it up for the thrust step, as it were. Not obvious in such a diagram, of course, is that ATP-regenerating systems maintain the chemical potential of ADP at a low level, thus facilitating its desorption from myosin and further aiding the thrust step. [Thoughts similar to these are independently discussed by K. Hotta and A. F. Huxley.]

In the foregoing narrative I have emphasized harmonious results in an effort to develop a coherent way of thinking about much of the contemporary research; however,

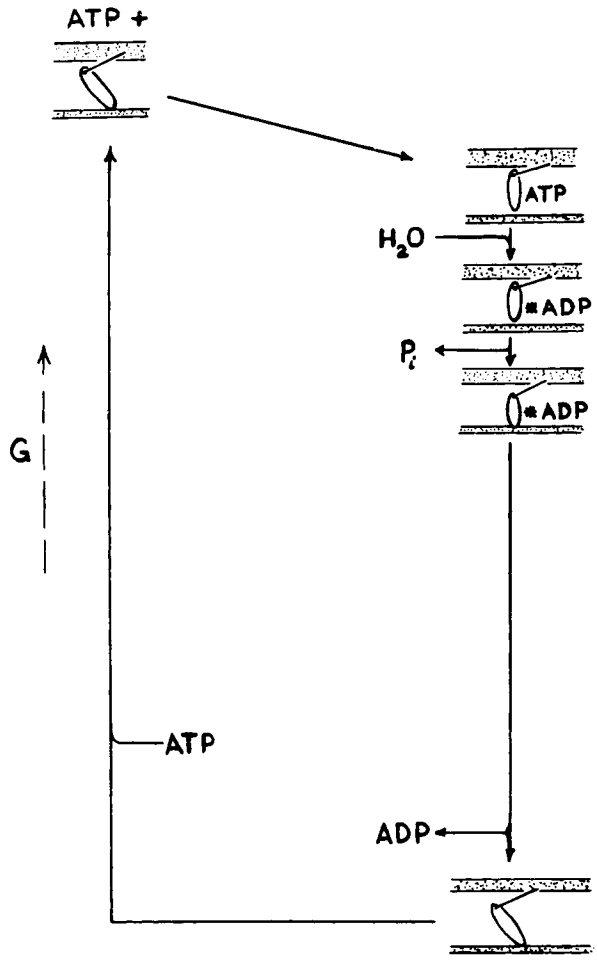

Fig. 1. Mechanochemical cycle of a crossbridge. Vertical height is intended to suggest the free energy of the system. At top left, ATP and the rigor arrangement are together but have not yet interacted. On the right hand side the nucleotide ligand on S1 is indicated next to the S1 ellipsoid. ADP refers to the "special" products complex mentioned in the text. The rigor arrangement of the lower right is converted to the upper left by adding the next ATP.

there are reported at this Conference observations by very reputable workers which either flatly contradict the foregoing story or are difficult to assimilate into it.

The duplex nature of myosin – revealed most emphatically by Slayter and Lowey's work (16) – may just lead to geometrical complexity, or even to concepts of molecular coordination (11, 17), but only if the two halves are exactly symmetrical. A. Martonosi

offered evidence that the two S1 moieties of the same molecule may not be alike [Martonosi discusses such evidence; S. A. Bernhard discusses the more general problem of symmetry in oligomeric enzymes].

Modern work has generally concluded that there is but one nucleotide binding site per S1, viz. the triphosphatase site, and our experience in affinity labeling with "thio-ITP" (18, 12, 17) suggests that it is this single site that gets labeled. Yount and his colleagues are reporting, however, that when the triphosphatase site is "covered" by "unsplittable ATP" (AMPPNP), affinity labeling with the disulfide of thio-ITP finds at least two additional sites on the same S1, and that occupancy of these additional sites exerts a controlling influence on the triphosphatase site [R. G. Yount et al. discuss this new work].

According to our story, the nucleotide bound to the muscle proteins in muscle at rest should be ADP (in the "special complex" and in actin), not ATP. M. Barany and T. Glonek report, however, that by using a novel method on live muscle fibers they detect creatine phosphate, and ATP, but not ADP. [Barany and Glonek discuss these matters.] I venture to interject that, aside from the identification of the bound nucleotide, the ^{31}P resonance method of these workers opens up spectacular possibilities of in vivo bioenergetic analysis.

Knowing the chemical changes (buffers included) attending a tetanus, and knowing the thermochemistry of the substances involved, one can calculate the total heat generated. Actual measurements, however, significantly exceed the calculation, thus admitting the possibility that there is an as yet unidentified energy source in muscle contraction. [M. K. Kretzschmar discusses this issue.]

Evan Eisenberg (19) has pointed out that when increasing actin is supplied to a myosin-ATP system, the steady-state ATPase rate (activated by actin) saturates at actin concentrations far less than those required totally to ligate the myosin [R. A. Mendelson et al. report that, in fact, the myosin is only about 30% ligated.] While this is not necessarily a discrepancy in what we have said, the result emphasizes that to write,

"special complex" + actin $\longrightarrow$,

is an oversimplification, for then the flux around the "bottle neck" (actin-induced increase in ATPase) should rise hand in hand with actin binding, which is contradicted by Eisenberg's result. Various explanations of the result have been offered by Eisenberg himself and by others; these have usually postulated the existence of still-undiscovered ATPase intermediates. An alternative may be to analogize with the Martonosi work (above) and to consider that if there is a very great interaction energy between an actin-occupied site and a "special complex," then a relatively low bound-actin concentration may disrupt the special complex and overcome the kinetic obstacle.

SECTION II

As has been mentioned in Section I, the S1 moiety of myosin undoubtedly suffers displacements in the manner of a rigid body, but probably no global distortions. It seems virtually certain, however, that such transitions as passing from M·ATP to the "special complex," desorbing ADP, or binding to actin are accompanied by local distortions ("conformational changes") which, though small in spatial extent, may have significant energetic consequences. Such local distortions have generally been detected by observing

environmental effects on "reporter groups" attached to various landmarks on S1. Analogous but less extensive studies have been made on actin, particularly on the G→F polymerization of actin [R. Cooke discusses the chemistry and energetics of this transition], although virtually nothing is known about distortions of actin on binding to myosin. Site-directed nucleotides have also been made, e.g., AMPPNP and thio-ITP, as already mentioned, but also fluorescent analogs such as "ϵ-ATP," and most recently, photo-affinity analogs of ATP [S. Jeng and R. J. Guillory, and elsewhere in the Conference, Boyd Haley, report such syntheses]; virtually all myosin site-directed analogs can also be incorporated into the nucleotide site of actin.

Jean Botts, using time-resolved fluorescence polarization decay methods, offers evidence that myosin bears a binding site for creatine kinase, an enzyme functionally related to myosin.

A general, though in practice sometimes difficult, way of charting S1 is to attach donor/acceptor probe pairs to various landmarks and then to examine for energy transfer between them, transfer being taken as evidence for proximity [R. P. Haugland outlines such an exploration of S1].

Local distortions of S1 created by the events of myosin ATPase seem best detected either as (a) perturbations of a nitro-oxyl ("spin label") probe attached to the reactive thiol of S1 [J. Gergely discusses this approach], or by (b) perturbations of an intrinsic tryptophane [A. G. Szent-Gyorgyi discusses this approach]. A variant of these methods is to test for solvent access to these probes using substances which destroy the probes, e.g., ascorbate reduction of spin labels or iodide quenching of tryptophane fluorescence [H. Onishi discusses this approach]. As already mentioned, this sort of work has been exceedingly useful for kinetics interpretations but has not yet yielded any "geographical" charts of S1 topography.

The other process studied widely by probe methods has been the activation of myosin ATPase. This phenomenon was discovered (20) by using myosin in 0.6 M KCl, activated by Ca^{2+}; eventually SH reagents and many other activators and inhibitors were discovered. Using Blum's (7) hypothesis that activation consists in the removal of products inhibition and that the products complex is stabilized by Mg^{2+}, several authors have formulated theories of myosin activation (e.g., 21). Research on this type of activation has always been inspired by the presumption that actin accelerated myosin ATPase by somewhat the same mechanism. However, actin activation is observable only at lower KCl concentration, say 0.15 M, in the presence of Mg^{2+}. Stone (22) made the important observation, however, that various modifiers also work at these more physiological conditions, and that, qualitatively, actin has the properties of other modifiers. This previous work has "set the stage" for ideas about activation that are more specific regarding the structure of the products complex, and which therefore further advance our knowledge of S1 topography [W. F. Harrington discusses a theory of actin activation].

The discovery and characterization of the light chains of myosin attached to S1 has not been paralleled by elucidation of their function, except in the case of Ca^{2+} activation of scallop myosin [A. G. Szent-Gyorgyi discusses the issue], which has been assigned to a particular chain as a result of very pretty experimentation.

The foregoing introduction to the papers has placed the various findings in a perspective which is obviously mine, and which should not be allowed to prejudice the

reader of individual contributions (one often finds jewels in grubby settings). As attenders will know, the Conference was strengthened and enlivened with a summation lecture by Sir Andrew Huxley, but at the time of this writing it is uncertain whether his words will filter through a typewriter and onto paper.

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Although it could not be presented at the conference there has been added to this compendium an important paper by L. Peller, dealing with the thermodynamics of binding of "one headed" (S1) and "two headed" (HMM) ligands of actin.

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