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On the molecular basis for chemomechanical energy transduction in muscle

(actin/myosin S-1)

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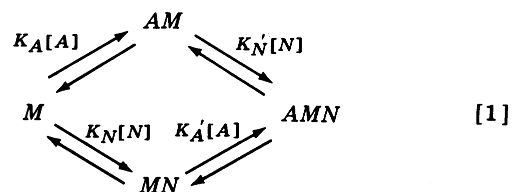
ABSTRACT **Herein it is developed that energy transduction in muscle is an activity of myosin S-1 and its ligands, actin (A) and nucleotide (N). S-1 shares with other molecular particles (e.g., hemoglobin) the property that binding events at one of its sites, the N site, influences binding events at a remote site, the A site (specifically, influences both the actin affinity and actin attachment angle at the A site). However, there is a crucial difference between S-1 and the better-known systems. Because the N site is enzymatic, it has a temporal sequence of occupants; this imposes a temporal sequence of actin attitudes—i.e., a sequence of mechanical events.**

The publication of a very interesting paper by Tregear and his associates (1) prompts us to set forth some thoughts that so far we have expressed only in symposia and research grant applications. The goal of explaining mechanochemical transduction in muscle (and other cells) has beckoned for a long time, and it is somewhat surprising that, now that the goal is becoming more distinct, little note has been taken of collective progress.

Beautiful and widely known researches have established that, in vertebrate skeletal muscle, myosin and actin filaments are somehow caused to translate relative to one another. And much-deepened knowledge of the myosin molecule has led to the realization that, although the myosin stem is firmly incorporated in the filament core, the S-1 and S-2 moieties (collectively, a "cross bridge") are a flexible appendage that radiates out to "touch" adjacent actin filaments. Much current research—including some of our own (2, 3)—is directed at verifying that, during activity, cross bridges can, and do, move repetitively, thus impulsively bringing about the relative translation of filaments. Together, these observations clarify how the contractile force is *transmitted* and *applied*, but not how it is *generated*.

Mechanochemical clues have come from other directions. One of these clues is that the myosin S-1 moiety, M (the distal part of the molecule having access to actin), has *interacting but distinct* sites—one for binding nucleotide (N), and one for binding actin (A). In developing the formal steady-state kinetics of actin-activated myosin ATPase we (4) simply guessed that this might be so. Subsequently, Bárány and Bárány (5) obtained evidence that the sites were indeed *distinct*. Our assumptions led us to include in our enzyme kinetic formulation the familiar "diamond" of relationships.



[1]

Szent-Györgyi's work (6) had long before implied that $K_A > K'_A$, and Kiely and Martonosi's work (7) implied that $K_N > K'_N$ (of course, $K_A/K'_A = K_N/K'_N$); so it is that *interaction* between sites occurs. But it is only recently that the Ks have actually been measured and that our notions about the sites have been strengthened by finding of ternary complexes, AMN (8, 9).

If we take N to be ATP, then the incorporation of Eqs. 1 into a steady-state kinetic scheme leads to a glorified Michaelis–Menten formulation such as we published (4). From experience with other enzymes, of course, it could be guessed that there is not just one intermediate, MN, in myosin ATPase but rather a sequence, such as MN_1, MN_2, etc. In the work of Trentham *et al.* (10), Taylor (11), and Tonomura (12), many of these species, MN_i, have been identified and put into proper sequence. In principle, a diamond of relationships, as in Eqs. 1, involving actin has to be assumed to exist for *each* N_i. Each diamond describes the binding reactions, and increasing i—e.g., $A^{(1)}MN_1 \rightarrow A^{(2)}MN_2 \rightarrow \cdots A^{(n)}MN_n$—describes the effect of chemical degradation of substrate.* Why we are superscripting A will be explained presently. The actual concentration $[AMN_i]$ depends on the numerical values of the Ks and of various rate constants (such numerical choices specify the most likely path through the scheme), but we wish to begin stressing qualitatively the feature that the thermodynamic instability of ATP, H_2O (relative to ADP, P_i), plus the fact that M is an ATPase, ensures that with time there will be a procession of ternary complexes, $A^{(1)}MN_1$, $A^{(2)}MN_2$, etc.

It seems to us that the other important clue in deducing mechanochemical transduction arose in Huxley and Simmons' (14) suggestion that in thrust the M of a cross bridge "rolls" on A. So preoccupied with cross-bridge operation has the muscle research community been that the most-discussed aspects of the Huxley–Simmons suggestion concern the usefulness of the M–(S-2) hinge (15), the possibility that energy may be stored

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Abbreviations: M, myosin S-1 moiety; N, nucleotide; A, actin.

* Matters of this kind are authoritatively treated in the book by Hill (13).

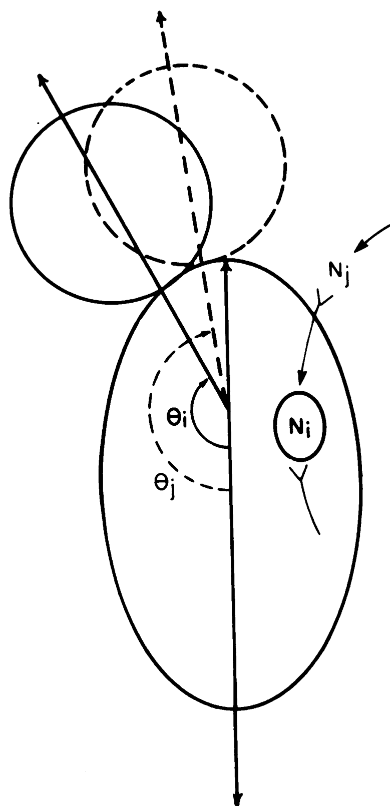

FIG. 1. Diagram of the transition from $A^{(i)}MN_i$ to $A^{(j)}MN_j$. The ellipse represents S-1 (M), and the circle represents an actin (A) monomer. For simplicity, the transition is depicted as a change in Θ, but see footnote †.

in an S-2-based elasticity, and so on. Nihei *et al.* (16) did do an experiment confirming a less-discussed implication—viz., that the contractile force arises at the M–A "interface." But from the "molecular" viewpoint, what now seems to us to have been the most interesting implication of Huxley and Simmons was the idea that *M and A can bind to each other at more than one angle.*

To bring this paper to its point we now wish to use a diagram showing the relationship of A to M in an A–M complex (Fig. 1). Such a diagram perforce assigns concrete shapes to both particles and may thus convey the impression that what we want to say depends on the correctness of the assumed shapes. This is not so. What we do assume here is that neither the A monomer nor the M moiety is an isotropic sphere, so that in describing the complex we can think of each particle as having an imbedded arrow. The natural "relational parameter" that we will discuss is the angle† between the arrows, Θ.

With the foregoing preliminaries in mind, and temporarily ignoring that N_i is just one of n sequential nucleotide species, we can take an "M view" of things, as in Fig. 1. Eqs. 1 have already emphasized for us that the affinities for A and N_i are interdependent and that what we have is a situation much like that discussed by hemoglobinologists—i.e., occupation of the N site by N_i causes information to be transmitted to the A site (presumably through peptide chain distortions), where its effect alters the affinity with which A is bound to the A site. The slight conceptual addition that we wish to emphasize is that from the chemical viewpoint, the binding of a ligand—whether large like A or small like N_i—is not fully characterized just by citing an affinity. Another characterizing parameter might be a relational angle†—for instance, the angle made by the normal to the adenine ring and the principal axis of M or Θ, the angle made by the principal axis of A and the principal axis of M. It is in fact the latter that we want to stress; we want to say that *corresponding to* N_i, *the angle between the principal axis of the complexed proteins assumes the value* Θ_i. Alternatively, this obligatory correspondence can be expressed by writing the "chemical formula" of the ternary complex as $A^{(i)}MN_i$, in which the superscript on A is simply a reminder about its angle of attachment to M.

This way of looking at things is no doubt obvious to chemists—it is quite possibly what Tregear and his associates (1) had in mind—but nonetheless it is essential to the mechanism of transduction. For now, we can think of sequence of *chemical* occupants of the N site (ATPase) as *coupled* (by means of polypeptide chain distortions?) to a sequence of the A site, $A^{(1)}MN_i \rightarrow A^{(2)}MN_2 \rightarrow$ etc.—i.e., to a mechanical sequence $\Theta_1 \rightarrow \Theta_2 \rightarrow$ etc. This view of things makes M the complete "molecular engine." Free energy is imparted to it by the binding of intact ATP (17, 18), and external work is performed by it when suitably loaded props are attached to it. The design of two distinct sites with an intervening transmission mechanism is identical to that elucidated in other macromolecules (notably hemoglobin) and its purpose is now clear. The *crucial* advance that converts it into an engine, however, is the variable nature of the N site occupancy, achieved through catalysis. As an engine it incorporates an admirable feature: because the catalyst of the fueling reaction (N site) and the moving part (A site) are tightly linked through the structure of M, chemistry is not allowed to proceed to any extent unless there is movement; probably this makes for a very high efficiency.

The foregoing thoughts are certainly prompted by the results of many workers, and perhaps they are only a rephrasing of thought entertained by others, but in one important respect we hope that their publication will be stimulating. To date, little of the work on M and A has focussed on the nature and structure of the binding sites, and no one has searched for a "transmission mechanism." Furthermore, although much attention has centered on attitudes of cross-bridges, no work has appeared examining whether A–M complexes can have different Θs. If the view of M that we have presented is roughly correct, then a new generation of research problems should lie ahead.

At various times Dr. John Gergely and Dr. Leonard Peller have offered comments that clarified our thinking on the subject of this paper. We also acknowledge with pleasure the friendly "needling" of Dr. David Green, who for years has insisted that students of muscle had not properly specified the transducer. We are grateful for research support from National Science Foundation Grant 22698 and National Heart, Lung and Blood Institute Grants 06285 and 16683. M.F.M. is a Career Investigator of the American Heart Association.

† *One* angle is mentioned here (and sketched in Fig. 1) just for simplicity. If A and M are constrained to "touch," then in general two angles will be minimally required to specify the relationship between their principal axes. We remark also that relational angles are necessary to characterize enzyme–substrate and enzyme–modifier complexes in general, but we believe that in the contractile system the angle is of special importance because elaborate and functionally important "props" are attached to both enzyme (M) and modifier (A).

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