The Mechanism of Adenosine Triphosphate Hydrolysis by Myosin

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Muscle contraction may be simulated in vitro by addition of Mg\(^2+\) and ATP to threads containing just two proteins, actin and myosin, in an aqueous medium. Shortening occurs by the interdigitation of the actin and myosin filaments, presumably driven by the cyclic attachment of cross-bridges, which are projections of the myosin filament containing the ATPase* site. The rationale for studying the myosin ATPase alone is to provide the basic framework for an understanding of the mechanism of modulation by actin. Further, in order to allow movement, the myosin cross-bridges must at some stage in their cycle detach from the actin and in this state they may undergo transitions that are common to the myosin ATPase mechanism. In the absence of actin the overall turnover of ATP by myosin is low (0.05 s\(^{-1}\)) and provides a model for resting muscle in which the actin–myosin interactions are blocked.

The evaluation of the myosin Mg\(^2+\)-dependent ATPase mechanism has greatly benefited from the application of transient-kinetic techniques (Trentham et al., 1976)

and has led to the following mechanism:

\[
\begin{align*}
M + ATP \rightleftharpoons^{1} & M\cdot ATP \rightleftharpoons^{2} M\cdot ATP \rightleftharpoons^{3} M^{**}\cdot ADP\cdot P_i \rightleftharpoons^{4} \\
M^{**}\cdot ADP\cdot P_i \rightleftharpoons^{5} & M\cdot ADP + P_i \rightleftharpoons^{6} M \cdot ADP \rightleftharpoons^{7} M + ADP
\end{align*}
\]

in which M represents a myosin site and \(k_{+i}, k_{-i}\) and \(K_i\) represent the forward and reverse rate constants and the equilibrium constant of the \(i\)th step. In a medium of 0.1 M-KCl, 5 mM-MgCl\(_2\) and 50 mM-Tris/HCl, pH8.0, at 21°C, \(K_1 = 4.5 \times 10^3\) M\(^{-1}\), \(k_{+2} = 400\) s\(^{-1}\), \(k_{-2} \leq 0.02\) s\(^{-1}\), \(K_2 = 9\), \(k_{+3} \geq 160\) s\(^{-1}\), \(k_{-3} = 0.06\) s\(^{-1}\), \(K_3 = 1.5 \times 10^{-3}\) M, \(k_{+4} = 1.4\) s\(^{-1}\), \(k_{-4} = 400\) s\(^{-1}\), \(K_4 = 2.7 \times 10^{-4}\) M (Bagshaw et al., 1974).

The key features of this mechanism include a rapid-cleavage step (3) (Lynn & Taylor, 1970), which precedes a rate-limiting isomerization (step 4), of the myosin–products complex (Trentham et al., 1972). Thus the predominant steady-state intermediate is \(M^{**}\cdot ADP\cdot P_i\) and gives rise to a transient in the total phosphate production (free + bound) on mixing an excess of ATP with myosin. The precise chemical nature of this intermediate is not known, but it is denoted in this way since ADP and \(P_i\) are released on quenching with acid. The binding and release of nucleotides are two-step processes and exemplify the idea of ‘induced fit’ (Koshland, 1958), in which a weak binary complex is first formed by collision, then on recognition an isomerization ensues that locks in the nucleotide. Evidence for this mechanism arises from the fact that the protein fluorescence change induced on nucleotide binding is a first-order process at high nucleotide concentration. In the case of ATP binding, the cleavage step follows immediately and the two processes are not well resolved under the conditions described for eqn. (1). However, the reaction of ADP and ATP analogues with myosin demonstrates that the fluorescence change is, at least in part, due to binding (Bagshaw et al., 1974).

The ready reversibility of the cleavage step (3) was first proposed from the following considerations (Bagshaw & Trentham, 1973). On mixing a molar excess of myosin

* Abbreviation: ATPase, adenosine triphosphatase.
sites with $[y^{32}P]ATP$ cleavage rapidly follows binding to give $M^{**}\cdot ADP\cdot P_i$. If cleavage was effectively irreversible $M^{*}\cdot ATP$ would never be observed since $k_{+3}\gg k_{+2}K_1$. [myosin] at the concentrations used. This is contrary to the experimental result in which it was found that on completion of binding 10% of the nucleotide was still ATP (i.e. arising from $M^{*}\cdot ATP$) and led to the estimation of $K_3$ as 9. The fact that the $[y^{32}P]ATP$ was tightly bound at this point was established by the failure of an added excess of unlabelled ATP to 'chase' it off.

The phosphate moiety of $M^{**}\cdot ADP\cdot P_i$ was known to undergo some kind of reversible hydrolysis reaction, since the product phosphate contained more than 3 oxygen atoms derived from the solvent water (Levy & Koshland, 1959). In addition it was established that the $P_i$ group of $M^{**}\cdot ADP\cdot P_i$ had already gained its water oxygen from the hydrolysis reaction, i.e. it was not a simple phosphorylated enzyme intermediate (Sartorelli et al., 1966). The relationship of this phenomenon, termed intermediate exchange, to ATP hydrolysis was not clear at that time, since no back incorporation of water oxygen atoms into the medium ATP was observed. However, intermediate exchange can be explained by the reversible incorporation of water at the cleavage step (3) of eqn. (1), as shown in eqn. (2) (Bagshaw & Trentham, 1973):

\[
\begin{align*}
M & \xrightarrow{K_1} M-R-P-O & & & & & & & & \xrightarrow{k_{+3}} M^{**}-R-O^*P-O \\
& & & & & & & & & \xrightarrow{H_2O^*} M^{**}-R-O^*P-O \\
R & & & & & & & & & \xrightarrow{k_{-3}} M^{**}-R-O^*P-O \\
& & & & & & & & & \xrightarrow{H_2O^*} M^{**}-R-O^*P-O \\
& & & & & & & & & \xrightarrow{k_{+4}} M^{**}-R-O^*P-O \\
& & & & & & & & & \xrightarrow{R} + M
\end{align*}
\]

where $R$ represents an ADP moiety and water oxygen atoms incorporated are marked with an asterisk. Such a mechanism predicts that oxygen-atom exchange between water and the terminal oxygen atoms of bound ATP (i.e. $M^{**}\cdot ATP$) will occur, although the incorporation of water oxygen atoms into the medium ATP will be negligible owing to the very slow dissociation controlled by $k_{-2}$. The fact that these predictions were realized (Bagshaw et al., 1975) demonstrates that a readily reversible hydrolysis step can account for intermediate exchange. Further studies by Trentham (1977) supported the proposal that hydrolysis is associated with cleavage, hence confirming the results of Sartorelli et al. (1966) that step 3 is not the formation of a phosphoenzyme (e.g. with a phosphoserine). In addition the formation of an acyl phosphate intermediate was ruled out.

The extent of exchange indicated that the oxygen atoms of the phosphate moiety are not equivalent, and it would appear reasonable that one atom is restricted owing to co-ordination with the myosin (Bagshaw et al., 1975; Shukla & Levy, 1977). However, the available evidence is also consistent with $M^{**}\cdot ADP\cdot P_i$ being a labile pentacovalent adduct of ATP and water, i.e. the $P_i$ and ADP moieties remain covalently linked by the $\beta-\gamma$-bridging oxygen atom, but are released on acid quenching. Such an $M^{**}\cdot ADP\cdot P_i$ intermediate might be distinguished from $M^{**}\cdot ADP\cdot P_i$, in which the ATP is actually cleaved, by the fact that the $\beta-\gamma$-bridging oxygen atom would not be free to exchange with the two non-bridging $\beta$-oxygen atoms. The appropriate $^{18}O$-labelling experiment is discussed by Trentham et al. (1976).

The reversibility of step 3 has been confirmed directly by the synthesis of ATP tightly bound to myosin from ADP and $P_i$, above the extremely low value expected from the
overall equilibrium constant for ATP hydrolysis (Mannherz et al., 1974). The synthesis of ATP is consistent with the rate and equilibrium constants of eqn. (1); nevertheless it is still very small owing to the unfavourable product-binding equilibria. More extensive ATP synthesis has been demonstrated by jumping the conditions (i.e. pH, ionic strength and temperature) during a single turnover of ATP hydrolysis so that the \( \text{M}^*\text{-ATP} \rightleftharpoons \text{M}^{**}\text{-ADP-P} \) equilibrium readjusts to the left (Inoue et al., 1974; Taylor, 1977). Thus the myosin ATPase provides a clear example of the Haldane relationship, which postulates that a differential affinity of substrates and products gives rise to a perturbed equilibrium constant for the enzymic reaction itself, i.e. hydrolysis step. Perhaps this phenomenon is used as a mechanism of ATP synthesis by other systems, hence it is feasible that the energy input is required to release tightly bound ATP, formed spontaneously from ADP and Pi (Boyer, 1975). Such a ‘conformational coupling’ does not lessen the problems of energy transduction, but shifts the role of pH gradients and membrane potentials to alterations of protein structure.

In the case of the myosin ATPase the tight binding of ATP is used to drive the dissociation of actomyosin cross-bridges during the cycle, and the actual hydrolysis step appears to occur on the dissociated myosin (see Weeds, 1977). Actin rebinds after hydrolysis to displace the products, thereby by-passing the slow step 4 of the myosin ATPase. Thus the \( \text{M}^*\text{-ATP} \rightleftharpoons \text{M}^{**}\text{-ADP-P} \) reaction is also a step in the actomyosin ATPase. The small free-energy change associated with this step results in the high efficiency of mechanical coupling, since the free-energy gradient of ATP hydrolysis is largely dissipated by attached states capable of doing useful work (Bagshaw & Trentham, 1973; Simmons & Hill, 1976).

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The Actin-Activated Adenosine Triphosphatase Activity of Myosin and its Proteolytic Subfragments

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Understanding of the mechanism of muscle contraction is based on the sliding-filament hypothesis (for reviews see Huxley, 1969; Huxley, 1974). The interdigitating thick and thin filaments of muscle are driven past one another by a cyclical movement of cross-bridges (myosin heads originating in the thick filaments and attached to actin monomers in the thin filaments). A single cross-bridge cycle contains a minimum of