Journal of Supramolecular Structure 3:333–337 (1975)

INTERMEDIATE COMPLEX OF ATP HYDROLYSIS AND SYNTHESIS BY MUSCLE PROTEINS

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Myosin catalyzed exchange between $^{32}P_i$ and ATP in reaction medium during its enzymatic hydrolysis of ATP only by a very small amount. Addition of actin increased to a great extent the rate of incorporation of $^{32}P_i$ in the presence of Mg. Glycerinated smooth muscle fibers also exhibited the ability to exchange $^{32}P_i$ and ATP upon the application of external force (repeated stretching and releasing). A schematic mechanism of the action of actin and external force on acceleration of $^{32}P_i$ incorporation is proposed and the importance of the M*-ADP complex for force generation is suggested.

INTRODUCTION

It has been suggested that the enzymatic site of myosin (heavy meromyosin HMM or subfragment 1 [S1]) undergoes a sequence of several activated states (M*, M**) during ATP hydrolysis (1–3). The process may involve conformational changes of a molecule due to the interactions between enzyme and substrate or products (4–6).

Among other approaches to exploring the nature of the intermediate complex and reaction mechanism of ATP hydrolysis by myosin, the isotope exchange technique provides valuable information. Experiments on the incorporation of ^{18}O of water into phosphate produced from ATP (intermediate exchange, [7]) and exchange between phosphate–^{18}O and water medium (medium exchange [8]) during ATP hydrolysis by myosin revealed that the hydrolytic step is reversible and not rate limiting in the overall reaction. Our previous work (9) shows that $^{32}P_i$ in the reaction medium is incorporated into ATP by actomyosin or myofibrils, suggesting that the M-ADP complex is an important intermediary for the mechanochemical coupling action of muscle proteins. Recently, Wolcott and Boyer (10) recognized that myosin, as well as HMM, could catalyze the $^{32}P_i$-ATP exchange even when present in small amounts, and that actin accelerated this exchange by stimulating dissociation of bound ATP.

It is important that the contractile system correlate an activated intermediate of protein(s) to the contractile cycle executed by a crossbridge, i.e. force generation. To clarify the mechanism of coupling between each step of ATP hydrolysis and energy transduction by muscle proteins, the effect of actin on myosin-catalyzed $^{32}P_i$-ATP exchange and that of external force on muscle fibers were investigated.

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MATERIALS AND METHODS

Preparation of myosin and actin was carried out by the usual method described in the previous report (9). Taenia coli isolated from rabbit intestine was glycerinated in 50% glycerol, buffered with 0.02 M histidine, pH 7.0, and stored at $-20^{\circ}C$ for 1 month. A small strip of glycerinated muscle (20 × 2 mm) was subjected to repeated stretch and release by the application of a square pulse (2–mm displacement) at various frequencies (1–5 Hz). The medium contained 1 mM Mg^{2+} and P_i ($^{32}P_i$) in 0.02 M histidine, pH 7.0.

Measurement of ATPase activity and analysis of $^{32}P_i$ and ATP were performed by the same procedures as in a previous experiment (9).

RESULTS

Actin Acceleration of Myosin ATPase Activity and $^{32}P_i$ Incorporation into ATP

Myosin ATPase activity in 0.02 M histidine in the presence of 1 mM Mg^{2+} was very low. Addition of actin greatly accelerated the rate of hydrolysis by a factor of more than 10, especially in the early period of hydrolysis. When $^{32}P_i$ was present in the medium, incorporation of $^{32}P_i$ into ATP was increased by the addition of actin, as shown in Fig. 1. The maximum rate of ATP^{32} formation was achieved by the addition of 2 moles of actin per mole of myosin. Actin alone (0.5 mg/ml) did not catalyze $^{32}P_i$ incorporation in any measurable degree. This excludes the possibility that the acceleration of the reaction is due to contamination by other enzyme(s) in the preparation of actin.

Incorporation of $^{32}P_i$ by Muscle Fiber

A strip of glycerinated taenia coli smooth muscle was used because, (a) it was easy to stretch and was mechanically strong, and (b) the fibers maintained a constant, steady ATPase activity for long periods of time, a quality which is required for the production of detectable amounts of ATP^{32}.

A diagram showing elution from the medium after reaction is shown in Fig. 2. Obviously, the glycerinated fibers contained active enzymes other than myosin ATPase. A large UV absorption peak which did not correspond to those of ATP and ADP appeared. Nevertheless, only a negligible amount of $^{32}P_i$ was incorporated into ATP when the muscle fibers were not stretched, while repeated stretching and releasing clearly stimulated the $^{32}P_i$ incorporation. These measurements were not accurate enough to enable estimation of the amount of exchange per unit proteins as a function of applied external force. However, the results were reproducible at least qualitatively when different preparations of fibers were used.

DISCUSSIONS

The results obtained in these experiments confirmed our previous report (9), and that of Ulbich and Rüegg (11), who have demonstrated the stretch acceleration of $^{32}P_i$ incorporation by insect muscle fibers.

Recently, Bagshaw and Trentham (6) and Mannhertz et al. (12) recognized ATP synthesis from ADP and P_i by S1 and obtained the rate constants associated with the backward reaction, suggesting that the rate-limiting steps for forward (ATP hydrolysis) and backward (ATP systhesis) reactions would be the degradation of M*-ADP and the

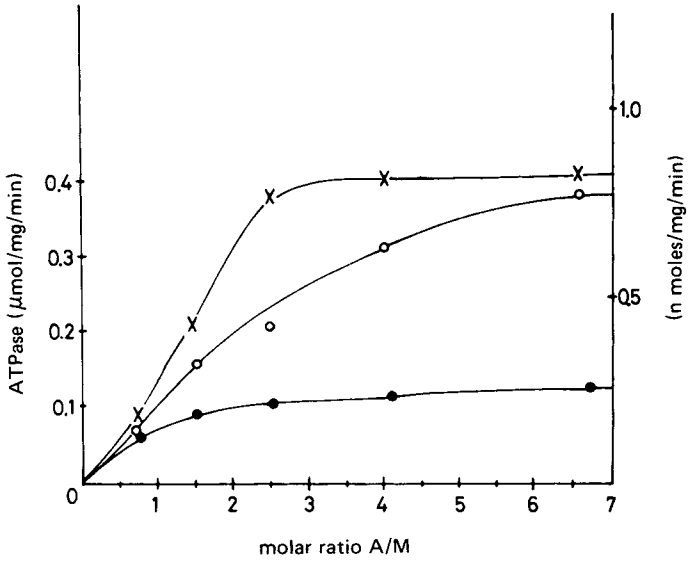

Fig. 1. ATPase activity and $^{32}P_i$ incorporation into ATP catalyzed by myosin and actomyosin. The concentration of myosin was kept constant (0.6 mg/ml) and various molar ratios of actin were added. Initial and steady ATPase activity indicates the average rate of hydrolysis during the initial 15 sec and that of the steady state, respectively. ○, initial ATPase; ●, steady state ATPase; X, incorporation of $^{32}P_i$ into ATP.

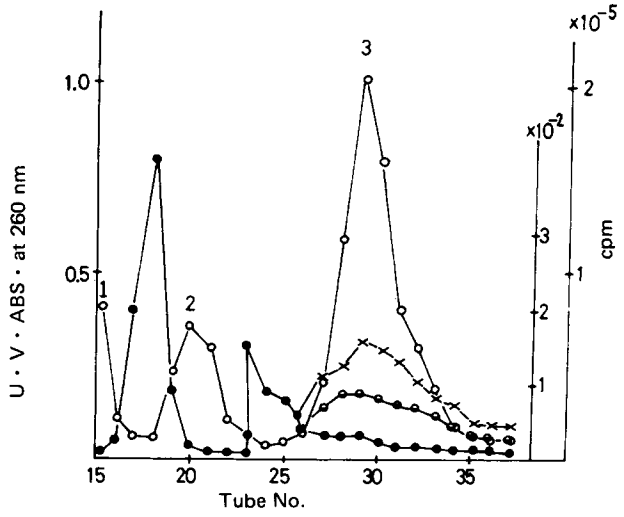

Fig. 2. $^{32}P_i$ Incorporation into ATP by glycerinated muscle fibers. Elution diagram from Dowex-1 column. ○, UV absorption at 260 nm. Peaks 1, 2, and 3 correspond to AMP, ADP, and ATP, respectively. ●, Radioactivity from $^{32}P_i$. Scale at ATP peak is expanded. ●, Control. Repeated stretch and release, ◒ at 2 Hz, and × at 5 Hz, respectively.

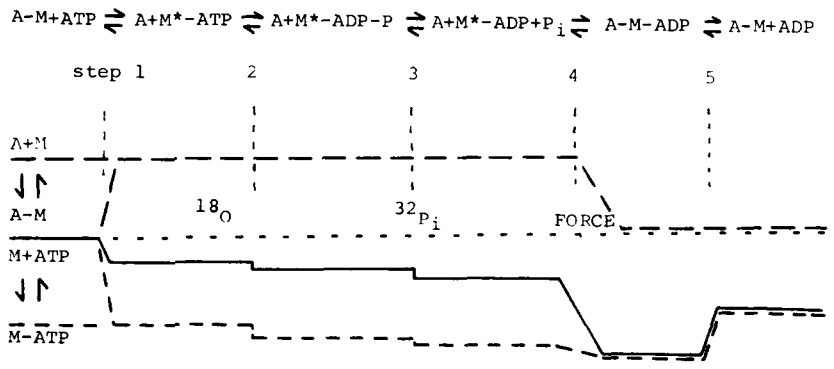

Fig. 3. Simplified schematic representation of energy level of the enzyme-substrate or enzyme-product(s) complex. — — M, A; - - - - - M, ATP, or ADP, P; ——— complete system. ^{18}O exchange and $^{32}P_i$ exchange at steps 2 and 3, respectively. Force generation is coupled with step 4.

dissociation of M*-ATP, respectively. Wolcott and Boyer (10) emphasized that the most important free energy changes involving protein conformation change (energy transduction) in the overall reaction occur at the step of binding ATP to myosin, and that actin stimulates $^{32}P_i$-ATP exchange by increasing the dissociation of bound ATP. However, if we assume that the free energy drop induced by ATP binding to myosin is a major source of chemical energy for muscle contraction, it is difficult to visualize how the energy-rich phosphate bond cleavage is coupled with force generation.

Since transformation of chemical energy to mechanical energy can be achieved only by interaction between myosin and actin, the effect of actin on the enzymatic action of myosin should be considered. A simplified scheme of ATP hydrolysis by myosin in the presence of actin can be illustrated as a diagram (Fig. 3). A, M, and M* (M* and M** in references 5 and 6) iepresent actin, myosin, and the activated state of myosin, respectively. The formation of M*-ATP may cause a large free energy drop, but at the same time, actin and myosin dissociate, which requires a considerable amount of energy. Thus, the actual free energy change of the whole system during step 1 should be relatively small. The differences of energy levels between subsequent steps of the reaction are small, so that this state may be maintained until at the step of degradation of M*-ADP. Apparently, the largest free energy change takes place at step 4. ^{18}O exchange and $^{32}P_i$-ATP exchange occur at steps 2 and 3, respectively. Actin accelerates the dissociation of the enzyme-substrate (step 1) and enzyme-products complex (step 5 and also probably step 4), increasing the rate of reaction in backward (step 1) and forward (steps 4 and 5) directions.

According to the scheme shown here, step 4 (M*-ADP ⇌ M-ADP) should couple with force generation (crossbridge movement). Mechanical energy (stretch and release) externally applied to muscle fibers may encourage the backward movement of crossbridges, modifying the reaction rate of step 4 in favor of the reverse direction. Then, the backwards reaction at steps 2 and 3 may be facilitated by the increment of the intermediate complex, M*-ADP. The fact that the stretching and releasing of mucle fibers increases $^{32}P_i$ incorporation into ATP is certainly supported by the scheme proposed here.

ACKNOWLEDGMENTS

The author wishes to express his thanks to Professor M.F. Morales of the University of California for his valuable suggestions and discussions.

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