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NOTE ON THE NATURE OF THE ACTIVE STATE OF THE ACTOMYOSIN-ADENOSINE TRIPHOSPHATE SYSTEM

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On the basis of the analysis of various experimental facts, in the previous papers1-5 the following scheme has been proposed as the reaction mechanism of the interaction between actomyosin and adenosine triphosphate (ATP),

\[
\begin{align*}
\text{M} + \text{S} & \rightarrow \text{M*S} \\
\text{M + P} & \rightarrow \text{S} \\
\text{M + S} & \rightarrow \text{M*} \text{S} \rightarrow \text{M*} \text{S} \rightarrow \text{M*} + \text{P}.
\end{align*}
\]

That is, ATP (S) binds on the ATPase active site of actomyosin (M) and the physical state of the actomyosin-ATP complex thus formed is changed by a reaction (M*S→M*S) which is accelerated in the presence of Mg++. The subsequent ATPase action brings about the formation of the active state of actomyosin (M*) and the recovery step in which guanidine kinase might play a predominant role closes this cyclic process. In the present note, which is a supplement to the previous paper,6 some discussions on the molecular nature of the active state and the reaction mechanism of the recovery will be presented on the basis of several recent experiments.

As was suggested by the author's group,7,8,9 guanidine kinase is an indispensable factor for the recovery from the physically changed state. As is well known, Mg++ is a necessary cofactor of guanidine kinase.10 Nevertheless, the recovery can be observed immediately after the addition of a chelate compound, EDTA, on the changed actomyosin.11 This result seems to imply that EDTA

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2 Y. Tonomura, this Journal 4, 87 (1956).
5 Y. Tonomura, K. Yagi and H. Matsumiya, Arch. Biochem. and Biophys. 64, 466 (1956).
7 Y. Tonomura and S. Kitagawa, unpublished observations.
prevents the formation of the active state and the observed recovery is the one from the inactive and physically changed state (M*) which does not need the kinase and divalent cation. MATSUMIYA et al.\textsuperscript{8) have shown that even when pyrophosphate added to 0.12 M KCl and 0.4 mM Mg\textsuperscript{2+} solution of actomyosin is decomposed by the subsequent addition of yeast pyrophosphatase, synaeresis cannot be brought about.

Based on his extensive experiments on contraction of muscle models, Weber\textsuperscript{9) has suggested that muscle contraction is induced by transference of the terminal phosphate of ATP to the contractile protein, accompanying breakdown of ATP. The above facts would seem to lend some supports on his suggestion, because EDAT is an inhibitor of transphosphorylation reaction and pyrophosphate is not capable of being phosphate donor in transphosphorylation. It is also interesting to note that the relaxing factors may be divided, on the basis of the above reaction scheme, into two categories, i.e., the one which inhibits the creation of the active state and the other which promotes the deactivation of the active state, and it may be suggested that EDTA\textsuperscript{10(11)} and PP\textsuperscript{12) belong to the former category and creatine kinase\textsuperscript{13) belongs to the latter.

In the preceeding reaction scheme, it was presumed that the active and physically changed state of actomyosin, created by the ATPase action, recovers to the original one through only one step in which the kinase is participating. However, considering the fact that the rate of the recovery does not decrease so much after considerable deprivation of the kinase system, it appears to be much more valid to assume that the recovery process consists of two steps $\tilde{M} \rightarrow M^* \rightarrow M$ and the former step is non rate-limiting and requires guanidine kinase.\textsuperscript{*) That is, the life of $M^*$ may be sufficiently long compared with that of the active state $\tilde{M}^*$. Accordingly, the more pertinent reaction mechanism may be given as follows:

\textsuperscript{*) The recovery can be observed also in the actomyosin-ITP system (Ref. 14), in which ITP cannot be the substrate of guanidine kinase. This may indicate that the action of the kinase as the recovery factor is not enzymic. Alternatively this fact can be envisaged as resulting from the contamination of minute quantity of nucleoside diphosphokinase in actomyosin or the presence of ATP and ADP, as impurities, in the ITP sample.

Note on the Nature of the Active State of the Actomyosin-Adenosine Triphosphate System

\[ M + P \]

\[ M + S \rightleftharpoons MS \rightarrow M*S \rightarrow \tilde{M}^* + P \]

\[ S + M^* \]

and in the presence of EDTA,

\[ M + P \]

\[ M + S \rightleftharpoons MS \rightarrow M*S \]

\[ S + M^* + P \]

As has been reported in one of the previous papers, the rate constant of the recovery can be estimated from the ATP quantity remaining at the initial stage of the recovery in the presence of Mg++. Thus the rate constant of the step, \( \tilde{M}^* \rightarrow M \), i.e., \( M^* \rightarrow M \), was found to be only \( 1/4.4 \) of the one of the ATPase reaction, \( M*S \rightarrow \tilde{M}^* + P \). Now the rate constant of the ATPase in the presence of Mg++ has been measured and found to be 0.31 sec\(^{-1}\). Then, the rate constant of the recovery becomes to be 1/14.1 sec\(^{-1}\).

As described above, Weber's suggestion on the transphosphorylation reaction of the actomyosin-ATP system is supported indirectly by our results. However, it would seem to be very difficult to afford the direct evidence of the transphosphorylation from ATP to actomyosin, because the life time of the active state (phosphorylated state of the protein) may be very short. Using labelled ADP\(^{15}\) and P\(^{16}\), Koshland, Budenstein and Kowalsky\(^{15}\) and the Ulbrechts\(^{16}\) have tested the transphosphorylation, but these authors have failed to obtain the successful verification of Weber's hypothesis.

16) G. Ulbrecht and M. Ulbrecht, cited in Ref. 9.