135. Ca\textsuperscript{2+}-binding Light Chain of Physarum Myosin Confers Inhibitory Ca\textsuperscript{2+}-sensitivity on Actin-myosin-ATP Interaction via Actin" \\

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(Communicated by Setsuro Ebashi, M. J. A., Dec. 12, 1985)

Introduction. We have shown that Ca\textsuperscript{2+} inhibits actin-myosin-ATP interaction of Physarum plasmodium by inhibiting the interaction,\textsuperscript{1,2,8} in sharp contrast with the activating effect of Ca\textsuperscript{2+} on the interaction of muscle,\textsuperscript{9} and proposed that this is the mechanism underlying the control of the cell motility of the plasmodium.

Under physiological conditions, which are estimated from the result of \textsuperscript{31}P-nuclear magnetic resonance of living plasmodia,\textsuperscript{10} the inhibitory Ca\textsuperscript{2+}-control is mediated by Ca\textsuperscript{2+}-binding to myosin.\textsuperscript{7} However, this myosin-linked regulation is hardly detectable under high Mg\textsuperscript{2+} conditions.\textsuperscript{2,4,6,7}

We isolated a fraction containing Ca\textsuperscript{2+}-dependent inhibitory factor from myosin B or natural actomyosin of Physarum.\textsuperscript{2} The addition of this fraction to actomyosin reconstituted of pure actin and myosin showed Ca\textsuperscript{2+}-inhibition under high Mg\textsuperscript{2+} conditions.\textsuperscript{2} As the factor bound to actin, the factor seemed to exert its regulatory action via actin.\textsuperscript{2} However, the active principle(s) has not yet been identified.

Under physiological conditions, myosin B is more sensitive to Ca\textsuperscript{2+} than reconstituted actomyosin.\textsuperscript{6} As myosin B contains both myosin and Ca\textsuperscript{2+}-dependent inhibitory factor, regulatory actions of Ca\textsuperscript{2+} on myosin B are mediated by both actin and myosin, i.e., Physarum actomyosin system is subjected to dual regulation.\textsuperscript{6}

In this paper, we isolate a fraction enriched with Ca\textsuperscript{2+}-binding myosin light chain of 14,000 Mr, the fraction which confers the inhibitory Ca\textsuperscript{2+}-sensitivity on the actin-myosin-ATP interaction under high Mg\textsuperscript{2+}-conditions. This 14,000 Mr component, seemingly identical with the Ca\textsuperscript{2+}-binding light chain, is considered as Ca\textsuperscript{2+}-dependent inhibitory factor.

Materials and methods. Myosin was prepared from plasmodia of Physarum
A fraction enriched with Ca\textsuperscript{2+}-binding light chain (see Results) of 14,000 Mr was prepared from myosin by denaturing myosin heavy chain and then subjecting to ammonium sulfate fractionation.\textsuperscript{7} Actin was prepared from rabbit skeletal muscle.\textsuperscript{11,12} The purity of protein preparations was monitored by SDS polyacrylamide gel electrophoresis (SDS PAGE).\textsuperscript{13}

\textsuperscript{12}I-labeling of the Ca\textsuperscript{2+}-binding light chain was carried out in Bolton and Hunter reagent (Amersham). Actin-affinity column was prepared by conjugating F-actin with Ultrogel resin.\textsuperscript{14} \textsuperscript{45}Ca\textsuperscript{2+}-binding to the 14,000 Mr light chain was demonstrated on Western blot of SDS PAGE of myosin.\textsuperscript{15} The ATPase activity of myosin was measured using a pH stat at pH 7.50 at 25°C.\textsuperscript{16} The Ca\textsuperscript{2+}-concentration was calculated using apparent binding constant for Ca/EGTA of 2.5\times10\textsuperscript{7} M\textsuperscript{-1} at this pH.\textsuperscript{17,18} Protein concentrations were determined by the method of Lowry using bovine serum albumin as a standard.\textsuperscript{19}

A polyclonal antibody against the Ca\textsuperscript{2+}-binding light chain was raised with guinea pigs by injecting the light chain, which was obtained by purifying a fraction enriched with the light chain by the preparative SDS PAGE. A monoclonal antibody against the myosin heavy chain was produced by hybridoma formation\textsuperscript{20} using BALB/c mice immunized with the heavy chain purified by the preparative SDS PAGE of myosin.\textsuperscript{21} The young plasmodia just after differentiation from amoebae of the colonial strain\textsuperscript{10} were subjected to double staining with the antibodies against the light and heavy chains.\textsuperscript{22} Further details will be described elsewhere.

**Results.** The Mg-ATPase activity of *Physarum* myosin was clearly inhibited by Ca\textsuperscript{2+} in the presence of skeletal muscle actin (Table I, upper line), confirming the previous results\textsuperscript{4} that Ca\textsuperscript{2+} exerts its inhibitory effect through myosin.

<table>
<thead>
<tr>
<th>light chain (\mu\text{g/ml})</th>
<th>(\text{Mg}^{2+}) mM</th>
<th>KCl mM</th>
<th>ATPase activity (n mol/min/mg myosin)</th>
<th>EGTA</th>
<th>50(\mu\text{M}) Ca\textsuperscript{2+}</th>
<th>%(^o)</th>
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<td>0</td>
<td>3.5</td>
<td>20</td>
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<td>5</td>
<td>119.5</td>
<td>97.6</td>
<td>18.3</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>8.5</td>
<td>5</td>
<td>123.2</td>
<td>61.0</td>
<td>50.5</td>
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</tbody>
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\(^{o}\) 100 \times (ATPase in EGTA—ATPase in Ca\textsuperscript{2+})/(ATPase in EGTA). Actin-activated ATPase activities were determined in the solutions containing 1.5 mM ATP, 100 \(\mu\text{g/ml}\) of actin, 41 \(\mu\text{g/ml}\) of myosin, 0.1 mM Ca-EGTA buffer and specified concentrations of the light chain, Mg\textsuperscript{2+} and KCl shown in the table.

We examined which subunit of myosin should be responsible for its high affinity for Ca\textsuperscript{2+}; \textsuperscript{45}Ca radioactivity was detected in the 14,000 Mr band of Western blot of myosin (Fig. 1). This observation is compatible with that of Kessler *et al.*,\textsuperscript{23} and suggests that Ca\textsuperscript{2+} exerts its inhibitory action by binding to this Ca\textsuperscript{2+}-binding light chain.\textsuperscript{23}

The inhibitory effect of Ca\textsuperscript{2+} on the actin-activated ATPase activity of myosin was hardly observed when assayed in the presence of high Mg\textsuperscript{2+} concentrations (Table I, middle line) in accord with the previous results.\textsuperscript{2,4,6,7} However, the inhibitory effect of Ca\textsuperscript{2+} was restored upon addition of a fraction enriched with the Ca\textsuperscript{2+}-binding light chains (Table I, lower line).
The fraction was labeled by $^{125}$I and absorbed to the actin-affinity column at low ionic strength. The radioactivity was eluted from the column with a solution of a high ionic strength (Fig. 2). This observation strongly suggests that the Ca$^{2+}$-binding light chain binds also to actin.

The antibody against myosin heavy chain stained only peripheral region of the plasmodial cell (Fig. 4b), but that against Ca$^{2+}$-binding light chain, whose specificity was tested in Fig. 3, stained the whole cell (Fig. 4a). These observations indicate that a considerable part of the Ca$^{2+}$-binding light chain is not incorporated into myosin molecules.

Thus it is quite possible that, in addition to the Ca$^{2+}$-binding light chain incorporated into myosin, myosin B may contain another kind of Ca$^{2+}$-binding light chain which works as a Ca$^{2+}$-dependent inhibitory factor through actin. The latter light chain may be responsible for the Ca$^{2+}$-sensitivity of myosin B which
is higher than that of reconstituted actomyosin.6)

Discussion. This paper suggests that Physarum myosin B contains additional Ca²⁺-binding light chain which is not incorporated into myosin. The Ca²⁺-dependent inhibitory factor, which confers inhibitory Ca²⁺-sensitivity on actin-myosin-ATP interaction by binding to actin, was isolated from myosin B preparation by mild treatment that may not compel myosin to release its light chain.7) Thus the active principle appears to be the light chain itself which was contained in myosin B preparation but not in the myosin molecules.
So far the Ca\(^{2+}\)-control of actin-myosin-ATP interaction, whether it would be inhibitory\(^{1-8}\) or excitatory\(^{9,24}\) has been classified in two categories, i.e., myosin-linked\(^{24}\) and actin-linked\(^{9}\). The myosin-linked control seems responsible for the inhibitory Ca\(^{2+}\)-sensitivity of the interaction of Physarum.\(^{4-8}\) The present study has revealed that the Ca\(^{2+}\)-binding light chain exerts its regulatory action also through actin as a Ca\(^{2+}\)-dependent inhibitory factor. Thus the Ca\(^{2+}\)-control of this lower eukaryote assumes a new type of regulation, in which a single small Ca\(^{2+}\)-binding protein exhibits a dual control through both actin and myosin.

Acknowledgements. We thank Prof. Y. Nonomura and M. Furuya for their discussions, interest and support. Our thanks are also due to Prof. S. Ebashi, M. J. A., for reading the manuscript.

References