日本財団 助成事業 The Nippon Foundation

財団法人日中医学協会
2006年度共同研究等助成金－在留中国人研究者－報告書

2007 年　3 月　2 日

財団法人　日 中 医 学 協 会　御中

貴財団より助成金を受領して行った研究テーマについて報告いたします。

添付資料：研究報告書

中国人研究者名：張　彫　　印

指導責任者名：小濱　一弘　　職名：教　授

所属機関名：群馬大学大学院医学系研究科・臓器病態薬理学

所　在　地：〒371-8511 群馬県前橋市昭和町3-39-22

電話：027-220-7962　内線：ダイヤルイン

1．助成金額：600,000 円

2．研究テーマ

カルシウム感受性ミオシンの構造と機能-分子生物学的アプローチ－

3．成果の概要（100字程度）

フィザルムという下等有核生物のミオシンはCa^{2+}の結合によって活性に阻害を受けるが、このミオシンのcDNAをクローニングして、発現蛋白質として得ることができました。このミオシンにはモーター活性があり、Ca^{2+}の阻害を受けることが再現できた。

4．研究業績

(1) 学会における発表　□無　・　☑有（学会名・演題）

日本薬理学会：カルシウム感受性真性粘菌ミオシンIIの発現と性質

(2) 発表した論文　□無　・　☑有（雑誌名・題名）

Regulatory mechanisms of striated muscle contraction. Calcium inhibition of Physarum myosin as examined by the recombinant heavy mero-myosin. pp.257-264, Springer Verlag, 2006

-日中医学協会助成事業-

カルシウム感受性ミオシンの構造と機能-分子生物学的アプローチ-

研究者氏名　張　影
日本研究機関　群馬大学医学部臓器病態薬理学
指導責任者　小濱一弘　教授
共同研究者　川道穂津美, 中村彰男, 吉山伸司

要　旨

Myosin II is one of the typical motor proteins and is classified as non-regulated, phosphorylatable and Ca-binding myosins. *Physarum* and scallop myosin II belongs to Ca-binding one. However, Ca^{2+} works as an inhibitor for *Physarum* myosin and as an activator for scallop myosin. This similarity in the subunits composition has raised the question of what subunit determines the inhibitory and stimulatory effects of Ca^{2+}. Myosin II regulated by Ca-binding has not yet expressed as a recombinant protein. Here, we report the expression of physarum myosin II together with preliminary characterizations.

Key Words　motility assay, calcium, recombinant myosin Ⅱ, actin

緒　言

Plasmodia of Physarum polycephalum shows vigorous cytoplasmic streaming by changing direction every few minutes. This oscillatory streaming is regulated by Ca^{2+} and is thought to be driven by a conventional myosin . It has been known that the superprecipitation of actomyosin preparation or myosin B from the plasmodia to examine the effect of Ca^{2+}. It superprecipitated without requiring Ca^{2+}. When Ca^{2+} at μM level was present, the superprecipitation was inhibited. This calcium inhibition was quite the opposite of the superprecipitation of actomyosin from vertebrate muscles, and we expected that the inhibitory mode could be involved in the plant cytoplasmic streaming. With the finding of the diverse classes of unconventional myosin such as myosin I and V in vertebrate muscles, the inhibitory mode was shown to play a role in cell motility in both animal and plant kingdoms. In this case the myosins have calmodulin (CaM) as the light chains and are regulated by interaction of Ca^{2+} with CaM, which exerts an inhibitory effect on activity. Since of the findings of calcium inhibition in the plasmodia, efforts have been made to define the way in which Ca^{2+} regulates the actomyosin system, leading to the discovery that, while that Physarum myosin is the major site of action of Ca^{2+}, actin-linked regulation through actin-binding protein is involved in the inhibition. Further biochemical studies on the myosin-linked regulation showed that Ca^{2+} binding to the Ca-binding light chain (CaLC) inhibits the activity of Physarum myosin　Similar to Physarum myosin, mollusk scallop myosin belongs to the myosinII isoform family and the activity of scallop myosin is regulated by Ca^{2+}. However, the effect of Ca^{2+} on this myosin is in an opposite to the regulation of Physarum myosin; Ca^{2+} activates the activity. Because structure and function in relation to the regulation by Ca^{2+} are known better for scallop myosin than that of Physarum myosin. We adopted a strategy to compare the two myosins for a better understanding of Ca^{2+} regulation. As the first step to analyze how Ca^{2+} exerts a regulatory role on Physarum myosin through binding to CaLC, we tried to obtain recombinant myosin and heavy mero-myosin of Physarum myosin (HMM).

対象と方法

We used baculovirus expression system. *Sf*9 cells were infected with the virus constructs.

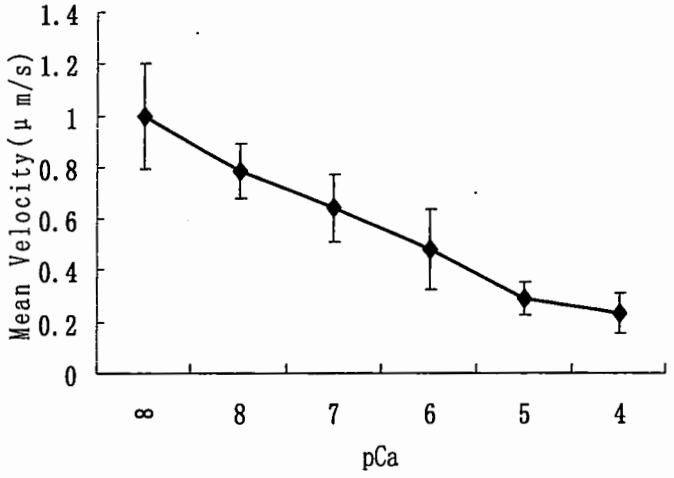
結　果

(1) Ca^{2+} inhibited sliding velocity of actin filaments propelled by recombinant full length myosin II and HMM of *Physarum* polycephalum , and as the concentration of calcium increased, the inhibition was stronger. But inhibition is stronger for full length myosin II, suggesting LMM maybe affected the sensitivity of myosin for Ca^{2+}.

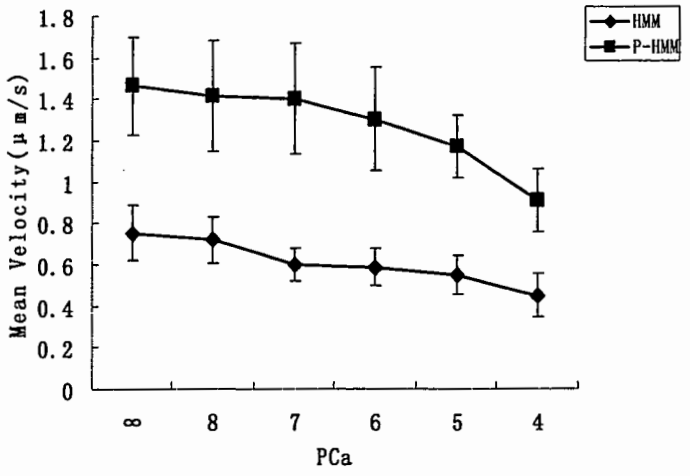
(2) The velocity of actin filaments caused by phosphorylated HMM was higher than unphosphorylated HMM, which agreed with the characters of Mg^{2+}-ATPase activity of myosin II before.

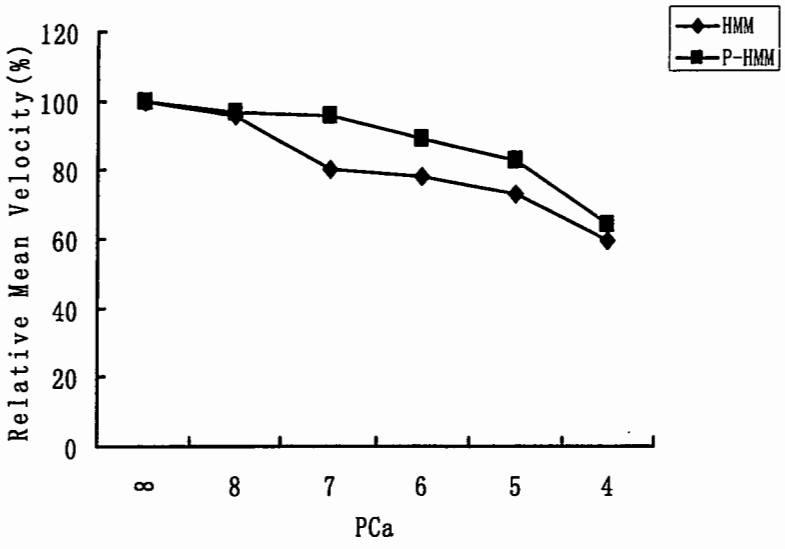
(3) Calcium could not impact the sliding velocity of actin filaments by mutant recombinant myosin II(it lacks calcium binding cite), suggesting that Ca^{2+} regulated the function of *physarum* myosin by binding with Ca^{2+}-binding light chain (CaLC).

1. The effect of calcium on the sliding velocity of actin-filaments on a glass surface coated with unphosphorylated physarum myosin.

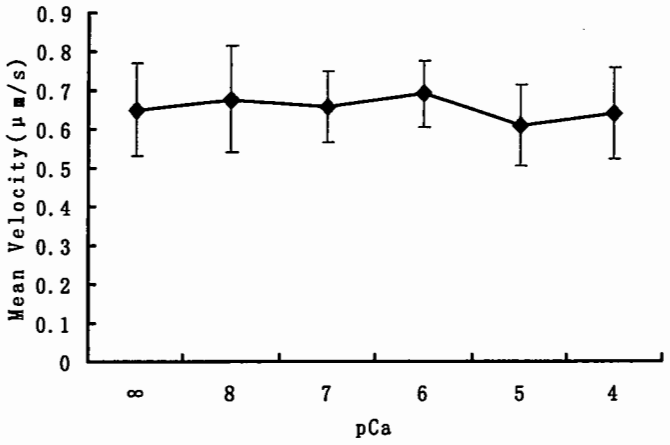


2. The effect of calcium on the sliding velocity of actin-filaments on a glass surface coated with unphosphorylated and phosphoryalted HMM.

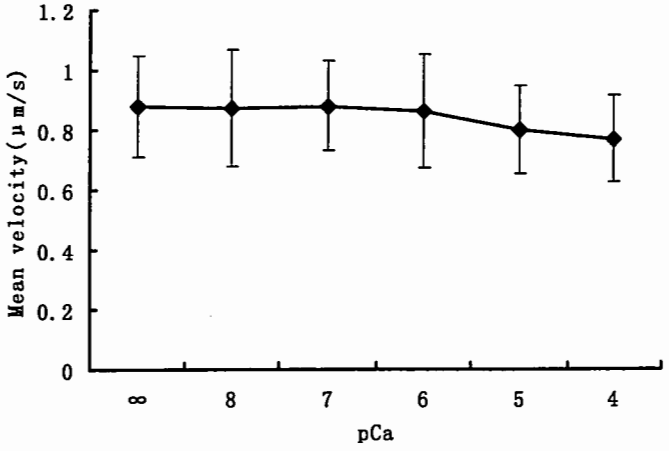




3. The effect of calcium on the movement of actin filaments on the mutant full length myosin-coated glass surface.



4. The effect of calcium on the sliding velocity of actin-filaments on a glass surface coated with mutant HMM.



考　察

To understand regulation of myosin II isoforms by Ca^{2+} comprehensively, i.e., including both activation and inhibition modes, we have expressed the hybrid HMM, it is consisted of physarum heavy chain, physarum regulatory light chain and scallop essential light chain, expecting the functional hybrid HMM. But we found that scallop essential light chain could not bind to physarum heavy chain.

参考文献

1. Kohama K, Kohama T, Kendrick-Jones J. The inhibitory Ca^{2+}-regulation of the actin-activaed Mg—ATPase activity of myosin from Physarum polycepharum plasmodia. J Biochem. 1986, 99, 1433-1446.

2. Hozumi Kawamichi. Characterization of recombinant Heavy Meromyosin of Physarum polycephalum. Kitakanto Med J. 2002, 52, 89-97.

3. Kohama K, Sohda M, Maruyama K, et al. Domain structure of Physarum myosin heavy chain. Protoplasma. 1988, 2, 37-47.

4. Kohama K, Kohno T, Okagaki T, et al. Role of actin in the myosin-linked Ca^{2+}-regulation of ATP-dependent interaction between actin and myosin of a lower eukayote, Physarum polycephalum. J Biochem. 1991, 110, 508-513.

5. Akio Nakamura and Kohama, K. Calcium regulation of the actin-myosin interaction of Physarum polycephalum. International Review of Cytology, 1999, 191, 53-98.

6. Ogihara, S., Ikebe, M., Takahashi, K. and Tonomura, Y. Requirement of phosphorylation of Physarum myosin heavy chain for thick filament formation, actin activation of Mg—ATPase activity, and Ca^{2+}-inhibitory superprecipitation. J Biochem. 1983, 93, 205-223.

7. Farkas, L., Andra´s Ma´lna´si-Csizmadia, Nakamura, A., Kohama, K. and La´szlo´ Nyitray. Localization and characterization of the inhibitory Ca^{2+}-binding site of Physarum polycephalum Myosin II. The Journal of Biological Chemistry. 2003, 278, 27399-27405.

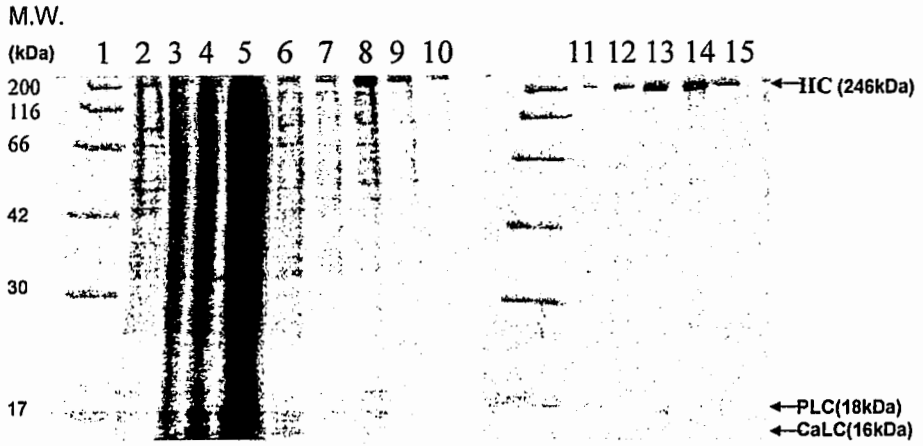
8. Kohama K. Heterogeneity of amino acid incorporation rate in adult skeletal muscle actin. J Biochem. 1980, 87, 997-999.

注:(1) 本研究は２００７年３月１６日に日本薬理学会で「カルシウム感受性真性粘菌ミオシン II の発現と性質」発表.

(2) Hozumi Kawamichi, **Ying Zhang**, Mizuki Hino, Akio Nakamura, Hideyuki Tanaka, Lászlo Farkas, Lászlo Nyitray, Kazuhiro Kohama. Calcium inhibition of Physarum myosin as examined by the recombinant heavy mero-myosin. In: Regulatory mechanisms of striated muscle contraction. Eds: S. Ebashi & I. Ohtsuki. pp257-264, Springer Verlag, (2006)

作成日　2007/03/01

1. SDS-PAGE of purification steps of recombinant myosin of Physarum polycephalum



1:Molecular weight marker
2:Sup. of *Sf*-9 uninfected homogenate
3: Ppt. of *Sf*-9 homogenate infected with both HC and LCs
4:Sup. of *Sf*-9 homogenate infected with both HC and LCs.
5: Sup. after the ultracentrifugation in the presence of ATP
6:effluence after using Ni-NTA column
7-10:Purified *physarum* myosin by using Ni-NTA column
11-15: Purified *physarum* myosin by using Superose ™6 column with HPLC

2. SDS-PAGE of purified recombinant HMM of Physarum polycephalum