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Rapid Communication

Interaction between myosin and a trace amount of caldesmon

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Caldesmon (CaD) is known as an actin binding protein. In this study, we proposed that a trace amount of caldesmon (TACD) could highly, efficiently, interact with myosin by producing a 'domino-like cascade' and characterized that TACD (lowest caldesmon/myosin molar ratio: 1/10,000) significantly increased precipitations and intrinsic tryptophan fluorescence intensity of myosin in both phosphorylated and unphosphorylated states compared to the base controls ($P < 0.01$). Actin-blocked TACD–myosin interaction, suggesting that it functioned as a negative regulator. Since CaD is not an enzyme, the *in vivo* significance of the highly efficient TACD–myosin interaction needs further investigation.

Keywords: caldesmon/domino-like cascade/myosin/phosphorylation/protein interaction.

Abbreviations: BSA, bovine serum albumin; CaD, caldesmon; CaM, calmodulin; CDPM, Ca^{2+}-CaM-dependent phosphorylation of myosin light chain; EGTA, ethylene glycol bis (2-aminoethyl ether) tetra acetic acid; MLCK, myosin light chain kinase; PMSF, phenylmethyl sulfonyl fluoride; TACD, trace amount of caldesmon.

The interaction between myosin and actin produces the contraction of smooth muscle. The contraction of smooth muscle is regulated by myosin-linked and actin-linked proteins. Myosin light chain kinase (MLCK), a myosin-linked protein, activates myosin ATPase by the phosphorylation of myosin regulatory light chain (*1*). On the other hand, caldesmon (CaD), an actin-linked protein, inhibits actin-activated myosin Mg^{2+}-ATPase activity via the interaction with F-actin (*2*, *3*).

Here, we used a co-sedimentation assay to observe the effect of a trace amount of caldesmon (TACD) myosin assembly, and used intrinsic fluorescence (*4–6*) intensity and myosin Mg^{2+}ATPase activity to measure the possible TACD–myosin interaction. We studied the influence of actin and bovine serum albumin (BSA) on TACD–myosin interaction respectively.

ATP, calmodulin (CaM), phenylmethyl sulfonyl fluoride (PMSF), dithiothreitol, BSA and ethylene glycol bis (2-aminoethyl ether) tetra acetic acid (EGTA) were purchased from Sigma-Aldrich (St Louis, MO, USA).

Myosin, CaD and MLCK were purified from fresh chicken gizzard smooth muscle as described previously (*7–9*). Preparation of Ca^{2+}-CaM dependently phosphorylated myosin (CDPM) by MLCK and determination of Mg^{2+}-ATPase activity were assayed as described (*10*, *11*). Both myosin (unphosphorylated) and CDPM were used in the assay.

The mixture of myosin (or CDPM) with CaD, or actin, or BSA was incubated in binding buffer containing 20 mM Tris–HCl (pH 7.5), 1 mM DTT, 5 mM $MgCl_2$, 60 mM KCl, 2 mM EGTA, 0.5 mM ATP at 25°C for 10 min followed by a 25-min centrifugation (140,000*g*) at 4°C. After centrifugation, the supernatant and pellet were applied to the SDS–PAGE. Our results indicated that the interaction between TACD (0.001 μM) and myosin (1 μM) was specific (Fig. 1A and B). When incubated with TACD, the precipitation of myosin was apparently increased; however, incubation with BSA (1 μM), a protein unrelated to myosin function, did not affect the precipitation of myosin. As a positive comparison, the precipitation of myosin was significantly increased in the presence of actin (1 μM). Furthermore, we observed that the precipitation of myosin increased by TACD was abolished in the presence of actin (1 μM). However, BSA could not abolish the interaction of TACD–myosin (data not shown).

Our results demonstrated that CaD (0.0001–10 μM) could efficiently interact with myosin (1 μM) and CDPM (1 μM) respectively in a dose-dependent manner; and even at the lowest CaD/myosin molar ratio (1/10,000), the sedimentations of myosin, i.e. the interactions of TACD with myosin and with CDPM were dramatically increased (Fig. 1C and D, $*P < 0.01$ versus control).

The intrinsic fluorescence due to tryptophan of myosin (1 μM) or CDPM (1 μM) was measured in a solution same as the solution for myosin phosphorylation and Mg^{2+}-ATPase activity determination using a spectrofluorometer, as previously described (*4*), with a slight modification (*12*). The excitation wavelength was 293 nm, and the emission spectrum was recorded from 310 nm to 380 nm. The emission intensities derived from TACD, BSA and actin were negligibly small, and if any, they were subtracted from that of myosin. Our results demonstrated that CaD at concentrations ranging from 0.0001 μM to 10 μM apparently increased the fluorescence intensity for both, myosin (Fig. 2A and D) and CDPM (Fig. 2B and E) in a dose-dependent manner. BSA (1 μM) did not influence and actin (1 μM) significantly increased the

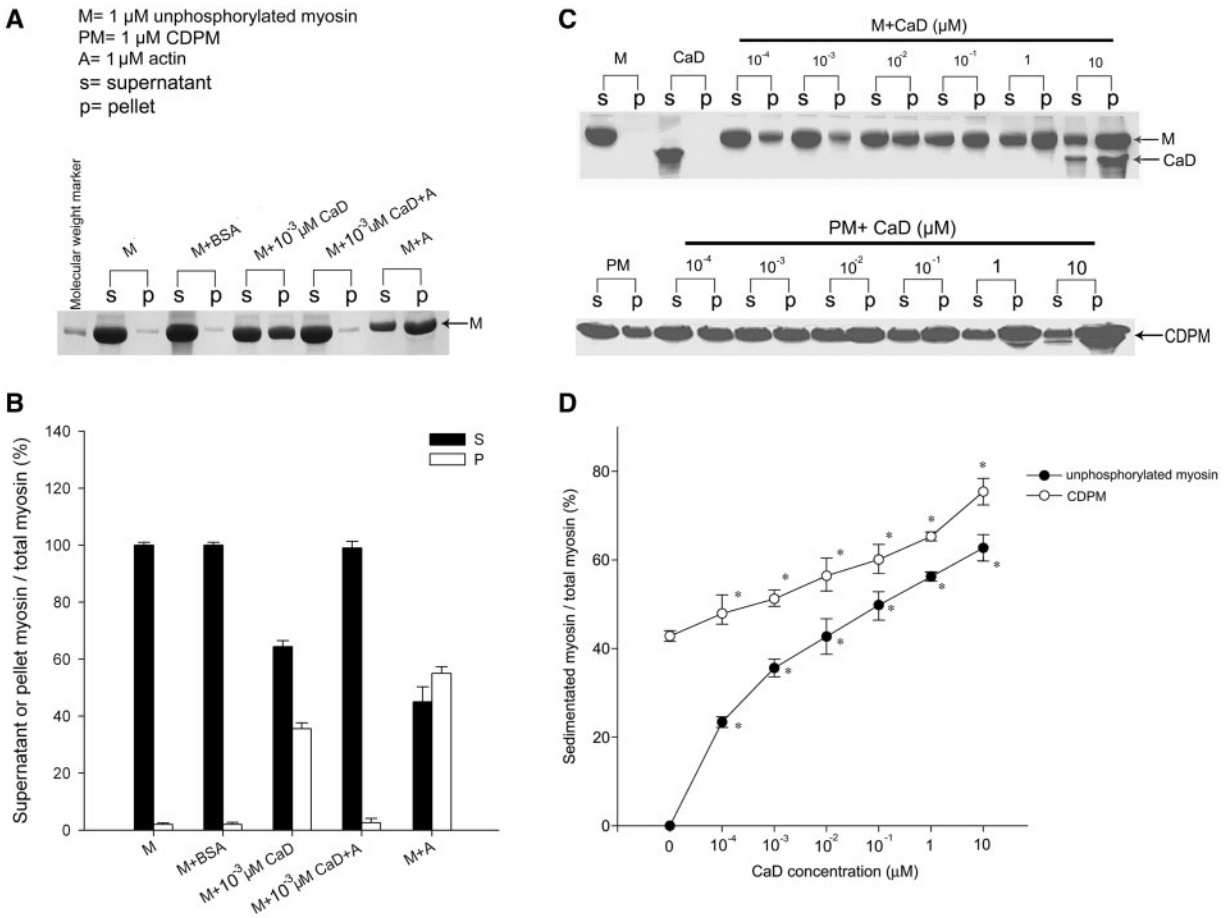

Fig. 1 Co-sedimentation assay of TACD–myosin. (A) Assessment of the specificity of TACD on myosin sedimentation by using SDS–PAGE. (B) The data was calculated from supernatant or pellet myosin/total myosin of four separate assays as panel A. (C) CaD at different concentrations acting on myosin and CDPM respectively. (D) The mean value of myosin sedimentation was calculated from four independent assays as panel C. The percentages of sedimented myosin (sedimented myosin/total myosin) were plotted against the concentration of CaD. $*P<0.01$ versus controls without CaD.

fluorescence intensity of myosin (Fig. 2C and F). However, actin (1 μM) significantly reduced the fluorescence intensity of myosin in the presence of TACD (0.0001 μM) (Fig. 2C and F). These observations were consistent with that of co-sedimentation assay, suggesting that TACD could induce the conformational change of myosin.

The measurement of Mg^{2+}-ATPase activity of myosin was previously prescribed (*10*, *11*). In the absence of actin, CaD at different concentrations (0.0004–0.4 μM) slightly, but significantly stimulated the Mg^{2+}-ATPase activities of myosin (0.4 μM), CDPM (0.4 μM) by MLCK, as compared with the corresponding controls (Fig. 3).

Based on our observation in the assay, we propose a domino-like cascade model for interaction between TACD and myosin. This model describes that TACD acting on myosin initiated the change of myosin conformation, probably leading to a cascade-like reaction in conformational changes for more myosin. The altered myosin conformation would be reflected in the properties of myosin precipitation and fluorescence intensity.

Our results supported domino-like cascade model for TACD–myosin interaction with the following characterization. First, compared to negative control BSA (1 μM), CaD, at a concentration ranging from 0.0001 μM to 10 μM, significantly increased the precipitation and fluorescence intensity of both myosin and CDPM, indicating the specificity of TACD–myosin interaction. Second, the TACD–myosin interaction was not observed in the presence of actin, i.e. both TACD-increased precipitation and the fluorescence intensity of myosin were abolished in the presence of actin (Fig. 1A and B; Fig. 2C and F). These results suggest that the highly efficient CaD–myosin interaction exists in the regulation of the myosin function when actin is dissociated from myosin. Finally, when the CaD/myosin ratio was over 1/1,000, CaD significantly, but only slightly increased Mg^{2+}-ATPase activities of myosin, implying the presence of loose relationship between myosin Mg^{2+}-ATPase activity and myosin assembly in terms of TACD.

Our results suggest that the complicated mechanisms involved in modulating the interaction of TACD and myosin remain to be elucidated, since the interaction was only partially characterized. For instance, in the presence of Ca^{2+}, TACD–myosin interaction was abolished in the presence of calmodulin or calmodulin + actin; and the interaction between TACD and

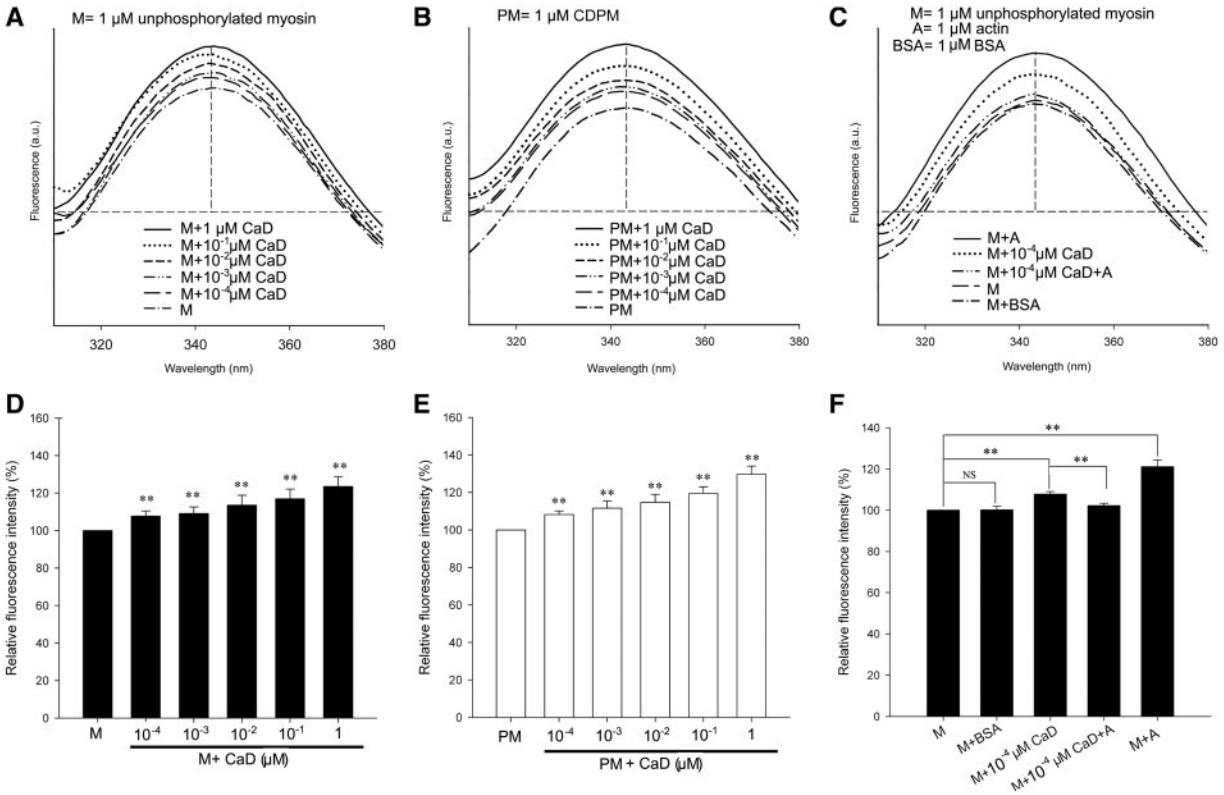

Fig. 2 TACD-induced myosin conformational change. (A and B) Fluorescence emission intensity of myosin and CDPM were measured in the absence and presence of CaD at different concentrations. (C) Fluorescence emission intensity of myosin in the absence and presence of TACD, actin and BSA respectivly. (D–F) The mean value of four assays of the fluorescence intensity as panels A, B and C respectively. Error bars: ±SE, $^{**}P<0.01$. NS, Not significant.

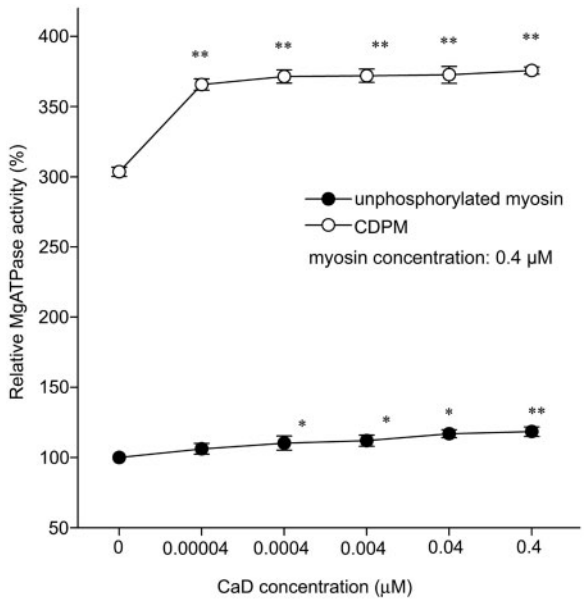

Fig. 3 TACD on Mg^{2+}-ATPase activities of myosin in different functional states ($\overline{X} \pm S$, $n=5$). The Mg^{2+}-ATPase activity of myosin without CaD (control) was calculated as 100% and other data were relative values compared to the control ($^{*}P<0.05$, $^{**}P<0.01$ versus the corresponding controls without CaD).

myosin could be abolished as well by other modulators regardless of the presence or absence of Ca^{2+} (data not shown). Further investigation is needed to uncover the exact three dimensional change of myosin after the interaction with TACD, and to reveal modulators involved in TACD–myosin interaction as well as the correlated mechanisms, and to understand the physiological role of TACD–myosin interaction.

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Conflict of interest

None declared.

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