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# CONFORMATIONAL CHANGES OF ACTIN INDUCED BY CALPONIN

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Calponin, an actin-linked regulatory protein in smooth muscle, caused a remarkable change in the fluorescence intensity of pyrene-labeled actin in the filamentous form. Calponin, an equimolar ratio to actin, decreased the fluorescence intensity of pyrene-labeled F-actin by some 60% to the level near monomeric actin. This change was partially reversed by $Ca^{2+}$, when calmodulin was present. Thus it appears that calponin causes conformational changes in actin molecules in an actin filament so as to inhibit their interactions with myosin. © 1992 Academic Press, Inc.

It has been established that there are both myosin- and actin-linked calcium regulations in vertebrate smooth muscle contraction. The role of caldesmon in the latter has been widely investigated (1-3). Calponin is another troponin-like protein that confers $Ca^{2+}$ sensitivity to smooth muscle actomyosin provided that calmodulin is present (4-6). Functional relationship between calponin and caldesmon has

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been recently examined by Dabrowska and her associates (7).

During the course of an investigation of the effect of calponin on actin polymerization, we have encountered with a peculiar phenomenon. Calponin markedly accelerated polymerization of actin when measured by flow birefringence, whereas calponin rather retarded polymerization when monitered by fluorescence intensity measurements using pyrene-labeled actin. However, it turned out that calponin greatly reduced fluorescence intensity of formed F-actin.

The present report describes a dramatic effect of calponin on the fluorescence intensity of pyrene-labeled F-actin suggesting conformation changes by calponin of actin molecules in the actin filament.

## MATERIALS AND METHODS

Preparation of proteins. Calponin was prepared from chicken gizzard smooth muscle according to Takahashi et al. (5) and purified by HPLC gel chromatography using TOSOH G2000SW column. As a byproduct of calponin purification, smooth muscle tropomyosin was prepared (8). Actin was isolated from rabbit skeletal muscle and purified by Sephadex G-150 chromatography. Calmodulin was purchased from Sigma.

Fluorescence measurements. Actin was labeled at Cys-374 with N-(1-pyrenyl) iodoacetamide following the method by Cooper et al. (9) based on the finding of Kouyama and Mihashi (10). Pyrene-labeled actin was mixed with unlabeled actin to give a final concentration of 10% labeled actin. Mixed G-actin was polymerized for 3 h in 0.1 M KCl, and 2 mM Tris buffer, pH 8.0. Fluorescence intensity was measured in a Hitachi 650-40 spectrofluorometer at 25°C and given in the same relative values throughout.

## RESULTS AND DISCUSSION

As revealed by a remarkable increase in fluorescence intensity due to a fluorescence dye binding to Cys-374 (9,

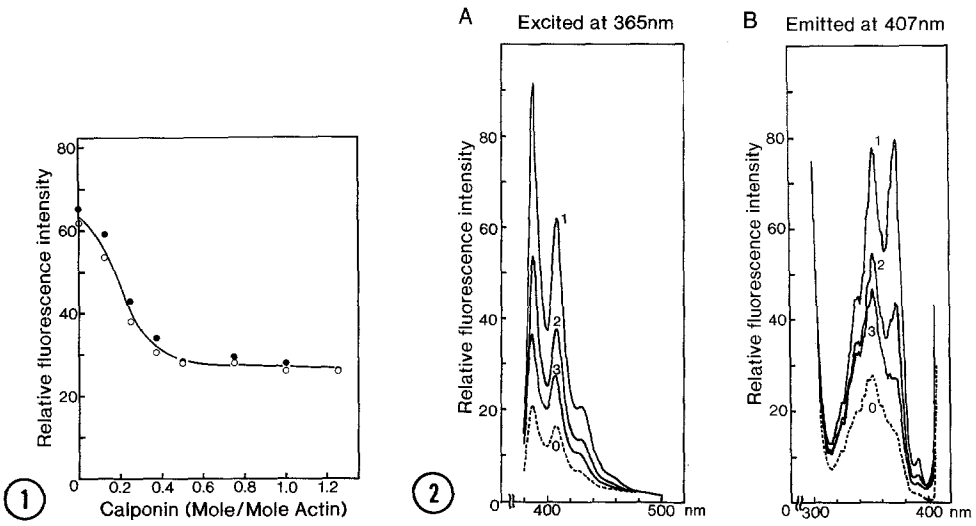


Figure 1. Effects of varied concentration of calponin on the fluorescence intensity of pyrene-labeled F-actin. F-actin, 0.3 mg/ml, tropomyosin, 0.07 mg/ml when added, 0.1 M KCl, 0.1 mM $CaCl_2$ and 2 mM Tris-HCl, pH 8.0. Incubated for 2 h at 25°C. Fluorescence intensity (excited at 365 nm; emitted at 407 nm) was measured 15 min after the addition of calponin. O, without tropomyosin; ●, with tropomyosin.

Figure 2. Effects of calponin on the fluorescence intensity of pyrene-labeled F-actin at varied excitation and emission wavelength. Conditions as in Fig. 1. A, excited at 365 nm; B, emitted at 407 nm. 0, G-actin; 1, F-actin; 2, F-actin with calponin, 0.25 molar ratio to actin; 3, F-actin with calponin, 0.50 molar ratio to actin.

10), conformational changes of an actin molecule occur when polymerized to form a double stranded filament. Fluorescence intensity of labeled F-actin excited at 365 nm and emitted at 407 nm was greatly reduced by the addition of calponin, as shown in Fig. 1. The decrease occurred regardless of the absence or presence of tropomyosin. The intensity maximally dropped by approximately 60% in the presence of calponin, 0.5

molar ratio to actin. This low level of fluorescence intensity was a little higher than that of G-actin. A half maximal change occurred in the presence of calponin, 0.2 molar ratio to actin. It is to be noted that calponin did not affect fluorescence intensity of G-actin.

The dependence of the changes in fluorescence intensity of F-actin on varied concentration of calponin is in good agreement with the binding curve of calponin to F-actin that is also not affected by tropomyosin (7). Thus, it is very likely that the conformational changes of actin monomer are caused by the binding of calponin to actin filament (one molecule of calponin per 3-4 actin monomers (4, 7)). It is of interest to note that caldesmon causes a drop of fluorescence intensity of pyrene-labeled F-actin; 15% in the absence and 25% in the presence of tropomyosin (11). The effect of calponin differs from that of caldesmon in that the decrease in fluorescence is much larger and tropomyosin does not affect.

The fluorescence intensity spectra emitted at varied wave length keeping excitation at 365 nm, and excited at varied wave length keeping emission at 407 nm are shown in Fig. 2A and B, respectively. Both spectra clearly show that the spectra of an F-actin solution became similar to those of a G-actin solution, when the amount of calponin added was increased. Thus it appears that conformation of an actin monomer in filament reverses toward that of free state, when calponin binds to the actin filament.

It has been established that the actin-myosin interaction in smooth muscle is inhibited by low $Ca^{2+}$ and activated by μM $Ca^{2+}$, when calponin, tropomyosin and calmodulin are present (6, 7). Therefore, we have tested

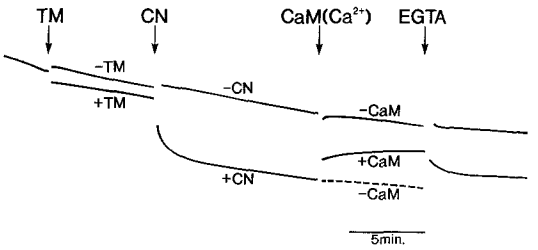


Figure 3. Effects of $Ca^{2+}$ on the fluorescence intensity of pyrene-labeled F-actin under the influence of calponin, tropomyosin and calmodulim. F-actin, 0.3 mg/ml, tropomyosin, 0.07 mg/ml, calponin, 0.034 mg/ml (1/7 molar ratio to actin), calmodulin, 0.17 mg/ml (10/7 molar ratio to actin), 0.1 M KCl, 0.1 mM $CaCl_2$ and 10 mM Tris-HCl, pH 8.0. When added, EGTA, 0.5 mM. Actin was polymerized for 2 h at 25°C. Fluorescence intensity (excited at 365 nm; emitted at 407 nm) was first measured for F-actin, then for 7 min in the presence of tropomyosin (TM), for 11 min (calponin (CN)), for 7 min (calmodulin (CaM)), and for 5 min (EGTA). Bar, 5 min.

whether $Ca^{2+}$ regulate the conformational changes of actin monomers in the calponin-tropomyosin-calmodulin-F-actin system. Fig. 3 shows a typical result. The fluorescence intensity of F-actin was not significantly affected by tropomyosin, but markedly decreased by calponin. After the addition of calmodulin in the presence of 0.1 mM $CaCl_2$, the intensity increased to the level 50% lower than that of control (without calmodulin). Further addition of 0.5 mM EGTA to reduce $Ca^{2+}$, a slight but significant decrease in fluorescence intensity occurred. These changes induced by $Ca^{2+}$ occurred without tropomyosin.

The present study indicates that the binding of calponin to actin filament changes the conformation of the COOH-terminal region (around Cys-374) of actin and this

effect may be correlated with the inhibitory effect of calponin on the actomyosin ATPase. Since calponin is localized along actin filament in cells (12, 13) and it is a normal component of native smooth muscle thin filament (14-16), it is probable that the conformational changes of actin by calponin reflect the physiological regulatory mechanism for smooth muscle contraction.

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