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Insight into the kinetics and the mode of the interaction between smooth muscle calponin and F-actin✪

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Kinetics of the smooth muscle calponin–F-actin interaction was studied by stopped-flow measurements of light scattering and fluorescence intensity of pyrene-labelled F-actin. The intensity and character of the changes in light scattering, and thus the mode of calponin binding to actin filaments leading to changes in their shape and bundling, depend on the molar ratio of the two proteins. Parallel measurements of pyrene-fluorescence quenching upon calponin binding revealed that intrinsic conformational changes in actin filaments are delayed relative to the binding process and are not markedly influenced by the mode of calponin binding. Bundling of actin filaments by calponin was not correlated with fluorescence changes and thus with alterations in the structure of actin filaments.

Calponin, originally discovered in chicken gizzard smooth muscle by Takahashi *et al.* (1986), is an actin-binging protein of molecular mass 32.3 kDa and dimensions of 16.2 × 2.6 nm, sufficient to span three monomers along the long pitch helix of actin filament (Stafford *et al.*, 1995). Three dimensional electron microscopic reconstructions suggest that calponin is located peripherally along the long pitch actin helix forming a shield-like density over subdomain 2 of actin and connects the axially adjacent actin monomers by binding to the upper and lower edges of subdomain 1 of each actin monomer (Hodgkinson *et al.*, 1997). It was initially suggested by El-Mezgueldi *et al.* (1992) that the region of calponin encompassing 145–182 amino-acid residues is implicated in actin-binding. Later on, it appeared that this region is actually composed of two distinct actin-binding sites on the

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calponin molecule: the first within amino acids 145–163, responsible for the inhibition of the actin-activated Mg-ATPase activity of myosin (El-Mezgueldi *et al.*, 1995; El-Mezgueldi *et al.*, 1996; Mino *et al.*, 1998) and the second, within amino acids 172–187, essential and sufficient for suppression of actin binding (Mino *et al.*, 1998) due to its phosphorylation by protein kinase C (Itoh *et al.*, 1995). The mechanism of the inhibition of actin-activated ATPase of myosin by calponin is a matter of controversy: some authors claim that calponin affects strong binding of actin to myosin (El-Mezgueldi & Marston, 1996) while others do not confirm this idea (Kołakowski *et al.*, 1997; Szymanski *et al.*, 1997).

It has been shown that calponin forms two types of complexes with actin: one at a molar ratio to actin up to 1:3–4, sufficient for almost full inhibition of the actomyosin ATPase activity (Kołakowski *et al.*, 1997; Szymanski *et al.*, 1997) and the other at a higher ratio leading to the formation of actin filament bundles (Kołakowski *et al.*, 1995; Kołakowski *et al.*, 1997; Lu *et al.*, 1995; Tang *et al.*, 1997). The ability of calponin to crosslink actin filaments implicates its structural role, i.e. formation, together with other actin-binding proteins (α-actinin, filamin), of the actin cytoskeleton (Leinweber *et al.*, 1999). Such dual role of calponin is supported by its distribution both in the contractile apparatus and cytoskeletal domain of smooth muscle cell (North *et al.*, 1994; Mabuchi *et al.*, 1996). It colocalizes with actin filaments (Walsh *et al.*, 1993) composed of smooth muscle α and γ isoforms as well as the cytoskeletal β isoform of actin (North *et al.*, 1994).

In order to gain further insight into the calponin–actin interaction we have studied its kinetics by monitoring changes in the light scattering and fluorescence intensity of pyrene-labelled F-actin using a stopped flow apparatus. The results show that the binding rate of calponin to actin filaments depends on the ratio of both proteins and always precedes the conformational changes in actin monomers.

EXPERIMENTAL PROCEDURES

Preparation of proteins. Calponin was prepared from chicken gizzard muscle according to Takahashi *et al.* (1986). Rabbit skeletal muscle actin was obtained by the method of Spudich & Watt (1971). The purity of both proteins was analysed by SDS/PAGE using 5–20% gradient mini-slabs by the method of Laemmli (1970). Protein concentration was determined by measuring ultraviolet absorbance with the following absorption coefficients and molecular-mass values: calponin, $A^{1\%}_{280}$ = 7.4 (Stafford *et al.*, 1995), 32 kDa; G-actin, $A^{1\%}_{290}$ = 6.3 (Houk & Ue, 1974), 42 kDa. Pyrene-labelled F-actin was prepared by labelling Cys-374 with *N*-(1-pyrenyl)iodoacetamide (Kouyama & Mihashi, 1981). The degree of labelling was at least 90% based on the absorbance of pyrenyl at 344 nm (Cooper *et al.*, 1983).

Stopped-flow fluorescence and light scattering measurements. The measurement of the time-course of calponin binding to actin was performed on a Spex Fluorolog spectrofluorometer equipped with Hi-Tech stopped-flow accessory (SFA-20) or on an SF-51 Hi-Tech stopped flow apparatus. The solutions containing various concentrations of calponin and 4 μM pyrene-labelled actin in 30 mM KCl, 2 mM $MgCl_2$, 0.5 mM EGTA, 0.5 mM 2-mercaptoethanol, 0.02% NaN_3 and 10 mM imidazole-HCl, pH 7, were loaded into the drive syringes. The samples were then pushed into the sample loop where equilibration at the experimental temperature lasted for 5–10 s before the reaction was initiated. Light scattering was monitored at 90° to the incident light at a 365 nm. Pyrenyl fluorescence, excited at 365 nm, was monitored at 397 nm. All concentrations refer to the reactants after they have been mixed.

Curve fitting routines. The curves were fitted using the nonlinear Levenberg-Marquardt algorithm. Changes in the light scattering or fluorescence clearly deviated from a single exponential fit. Therefore, fitting to a double ex-

ponential plot was performed according to equation:

$$y = A_o + A_1 \cdot e^{-(k_1 \cdot t)} + A_2 \cdot e^{-(k_2 \cdot t)}$$

where k_1 and k_2 are the observed rate constants and A_1 and A_2 are the corresponding amplitudes of the changes. The observed rate constants (k_{1obs} and k_{2obs}) were calculated from at least three traces. Typical traces are shown in the Figures. Simulation of the binding process was made using the KINSIM program (Barshop *et al.*, 1983).

RESULTS

The rate of complex formation between calponin and filamentous actin at various molar ratio and low ionic strength was measured by light scattering. Since the interaction of the elongated protein molecules with protein polymers affects not only the mass but also the shape (diameter) of the polymers (Cantor & Schimmel, 1980), the intensity of light scattering which is sensitive to the changes in those parameters appeared to be convenient in studying the mode of calponin binding to actin filaments. As shown in Fig. 1, the character of changes in light scattering depended on the calponin to actin molar ratio. At the ratio of 0.4 : 1 light scattering was gradually increased (Fig. 1A). The obtained curve was best described by a double exponential fit (k_{1obs} = 1.1 s^{-1} and k_{2obs} = 0.13 s^{-1}) since a single exponential plot did not fit the data adequately. In this case, calponin molecules were likely to be initially bound randomly through one or another actin-binding site along the actin filament and instantly were rearranged by utilising both actin-binding sites to the longitudinal position, which is thermodynamically favoured. A small increase in the actin filament diameter caused, in consequence, a small (about 7%) increase in the light scattering signal over that produced by actin filaments alone. Measurements of the rate of association of calponin with actin was complicated by bundling of actin filaments occurring at higher calponin concentrations (Fig. 1C) (Kołakowski *et al.*, 1995; 1997; Lu *et al.*, 1995; Tang *et al.*, 1997). Therefore, we estimated the association rate constants using the KINSIM program, on the assumption that binding was irreversible. The estimated rate of binding describes the lower limit of the velocity for this reaction. The simulation was made according to a simple binding scheme (1) or according to a two step binding Scheme (2).

$$Ac + Cap \xrightarrow{k} Ac{\cdot}Cap \qquad \text{Scheme 1}$$

$$Ac + Cap \xrightarrow{k_{1^*}} Ac{\cdot}Cap + 2ac \xrightarrow{k_{2^*}} Ac_3{\cdot}Cap$$

$k_{1^*} << k_{2^*}$, Ac – actin, Cap – calponin

Scheme 2

In Scheme 2, we assume that binding is followed by a very fast reorganization connected with additional binding of calponin to two actin molecules. This assumption leads to a better fit of the experimental data. The calculated association rate constant $k_{1^*} = 2 \times 10^5$ M^{-1} is in agreement with that estimated by Lu *et al.* (1995) by measurement of the rate of caldesmon dissociation from actin filaments after addition of calponin.

On increasing ratio of calponin to actin to 0.6:1 (still below the saturation level) the light scattering changes accompanying the binding of both proteins were much faster (< 0.5 s) and more pronounced (Fig. 1B). The character of the changes was typical for a multiphasic reaction (Cantor & Schimmel, 1980) and can be interpreted in terms of the two mode binding model proposed previously (Kołakowski *et al.*, 1997). The fast initial binding induced a marked (about 60%) enhancement of the light scattering, probably due to the mostly transverse orientation of calponin molecules with one site of attachment to actin filaments and a

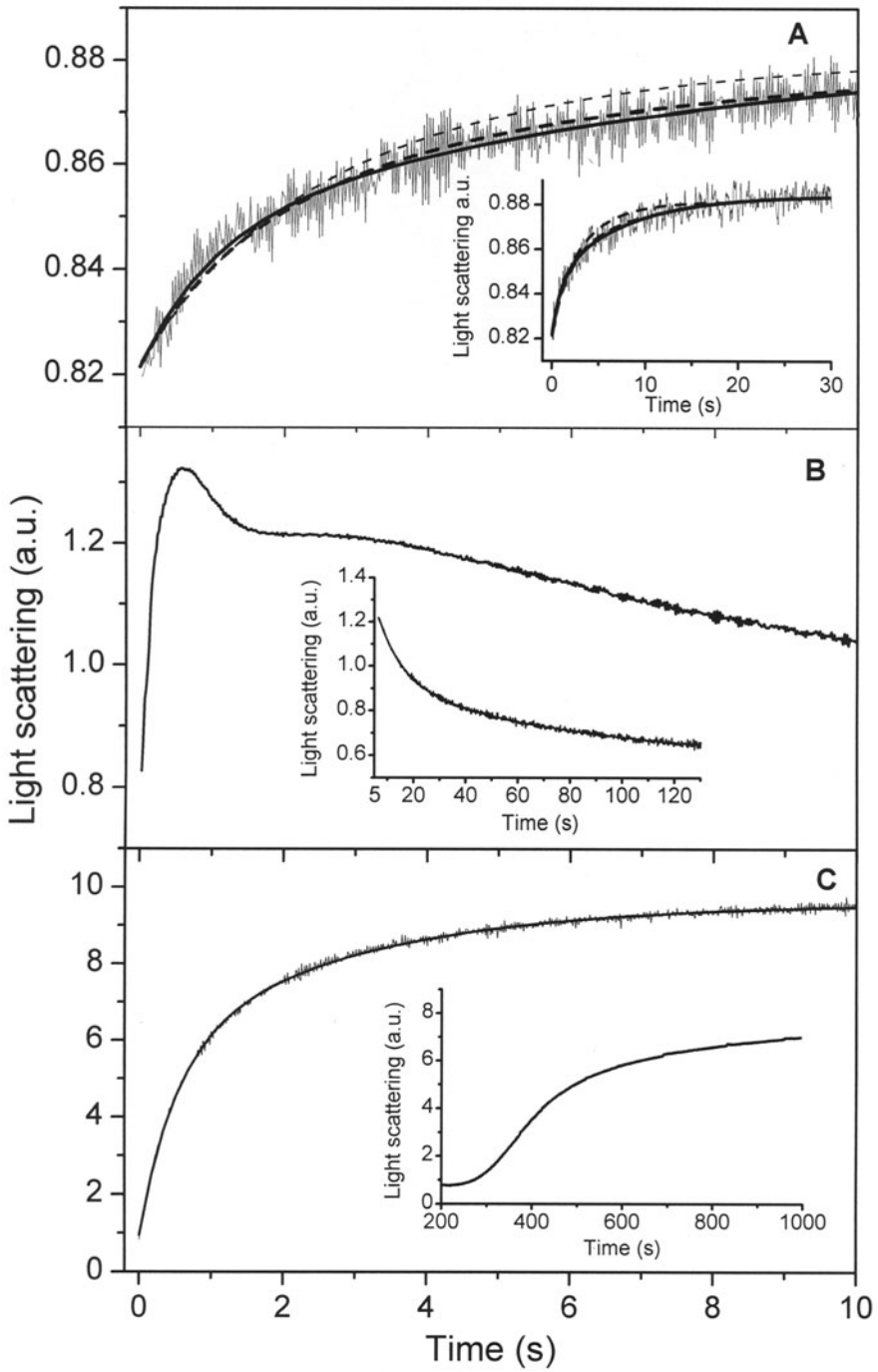

Figure 1. Changes in light scattering during the interaction of calponin with F-actin at various ratios of both proteins.

Samples contained various amounts of calponin and 2 μM F-actin in 30 mM KCl, 2 mM $MgCl_2$, 0.5 mM EGTA, 0.5 mM 2-mercaptoethanol, 0.02% NaN_3 and 10 mM imidazole-HCl, pH, 7.0. **A**. Binding of 0.8 μM calponin to actin. Inset shows the whole binding process. **B**. Binding of 1.2 μM calponin to actin and sequential rearrangement of calponin on actin filaments. Inset shows the slow rearrangement phase. **C**. Bundling of actin filaments by 1.6 μM calponin. Inset shows sequential long time interval view of the light scattering curve presented in B, corresponding to the bundling process. Bold lines represent double exponential fits. Corresponding rate constants and amplitudes are given in Table 1. The units on the ordinate axes are arbitrary, however, they are the same for all three panels. Dashed lines (A) represent a simulation of binding of calponin to actin according to scheme 1 with association rate constant $k_1 = 2 \times 10^5\ M^{-1}s^{-1}$ and bold dashed lines according to scheme 2 with association rate constant $k_{1^*} = 2 \times 10^5\ M^{-1}s^{-1}$. Measurements were performed at 20°C.

Table 1. Kinetic parameters of calponin binding to F-actin.

The relative changes, amplitudes and observed rate constants were calculated from the light scattering and the fluorescence curves using the procedure described in the Material and Methods section.

Calponin to actin ratio	Light scattering						Fluorescence		
	Binding			Rearrangement					
	Relative changes of light scattering	Rate constants		Relative changes of light scattering	Rate constants		Relative changes of fluorescence	Rate constants	
		k_{1obs}	k_{2obs}		k_{1obs}	k_{2obs}		k_{1obs}	k_{2obs}
mol/mol	(%)	(s^{-1})	(%)	(%)	(s^{-1})	(s^{-1})	(%)	(s^{-1})	(s^{-1})
0.4	7	1.1 ±0.15 (3)	0.13 ±0.02 (4)	–	–	–	–32	0.19 ±0.02 (–18)	0.02 ±0.002 (–14)
0.6	60	n.d.*	n.d.*	–46	0.10 ±0.01 (–20)	0.011 ±0.001 (–27)	–37	0.25 ±0.02 (–20)	0.026 ±0.003 (-17)
0.8	1050	1.9 ±0.2 (570)	0.36 ±0.05 (480)	–	–	–	–43	0.31 ±0.04 (–17)	0.03 ±0.004 (–26)

*The binding was followed by the fast first rearrangement step therefore it was impossible to calculate binding rate constants. Values in brackets represent amplitudes corresponding to rate constants (in %).

significant increase in their diameter. The calculation of the binding rate constant was, in this case, impossible due to the superimposition of the binding phase with a subsequent two-step decrease in the light scattering, which probably reflects the rearrangement of calponin molecules on the actin filament to the longitudinal position. First, an about 10% decrease in light scattering signal occured relatively quickly because there were still some unoccupied, free binding sites on the actin filaments. The following steps were very slow (k_{1obs} = 0.1 s^{-1}, k_{2obs} = 0.011 s^{-1}) (Table 1) due to steric hindrance and a probable requirement for the dissociation of calponin molecules from actin filaments, which seems to be rate limiting since the observed rate constant of this step was in the range of the dissociation rate constant (Lu *et al.*, 1995). Moreover, double exponential fitting suggests that calponin dissociated from the two different actin-binding sites. A small number of calponin molecules which preserved a transverse orientation caused, after a long lag phase (200 s), cooperative bundling of actin filaments manifested by a large increase in the light scattering (Fig. 1C, inset).

At the calponin to actin ratio of 0.8 : 1, when almost all actin monomers in the filament were saturated by calponin and its longitudinal arrangement on the filament was impossible, the number of calponin molecules in the transverse, bundling orientation was much higher. Therefore, as demonstrated by an almost 10-fold increase in light scattering signal, bundling of actin filaments appeared immediately (k_{1obs} = 1.9 s^{-1}) upon mixing of both proteins (Fig. 1C). This explains why, in this case, we could not distinguish between the binding and bundling phases. The amplitudes of changes in light scattering signal accompanying bundling were much higher than observed during binding of calponin to actin. Thus, the complicated double exponential character of the registered curve was probably caused by crosslinking and subsequent reorganisation of the actin filament network. The bundling process itself is likely to show complicated kinetics as the observed signal is a weighted sum from a heterogeneous popula-

tion, with larger bundles having a stronger scattering characteristics. Thus, the higher rate constants correspond to the rate of bundling.

To obtain information on the effect of calponin binding on the actin filament structure, in parallel with the rates of light scattering, the rates of fluorescence intensity changes of pyrene-labelled F-actin upon its interaction with calponin were measured. At all ratios of calponin to actin, the fluorescence intensity of pyrene-labelled actin dropped gradually, at a rate proportional to the ratio of both proteins (Fig. 2, Table 1). Fluorescence quenching for the ratio of calponin 0.4, 0.6 and 0.8 moles per actin monomer reached 32, 37 and 43%, respectively. The fluorescence quenching curves were best fitted by a double exponential equation. As shown by the fluorescence parameters (Table 1) the quenching is induced by two processes of comparable amplitude but with rates differing by a factor of 10. This probably reflects the binding of calponin molecules to actin filaments through distinct actin-binding sites.

Comparison of the time-course of the light scattering and fluorescence intensity changes showed that, at the lowest calponin to actin ratio, the fluorescence quenching rate was lower (k_{1obs} = 0.19 s^{-1}) than the rate of binding (k_{1obs} = 1.1 s^{-1}). Acceleration of the binding processes at a higher calponin ratio (0.6 : 1) and immediate rearrangement of calponin molecules on the actin filament prevented calculation of the binding rate constant. However, comparison of the curves presented in Fig. 1B and Fig. 2 (curve b) showed that, during the initial binding phase, the fluorescence intensity decreased by only 5%. Thus, the delay in the decrease of fluorescence intensity indicates that it has been caused not by calponin binding itself but by slow changes in the intrinsic structure of actin filaments induced by this binding. The bundling of actin filaments by calponin was not correlated with fluorescence quenching: at an intermediate ratios of calponin to actin monomer (0.6 : 1), the bundling followed, but at a higher ratio (0.8 : 1) it preceded the fluorescence quenching (Table 1). Nevertheless, the observation of

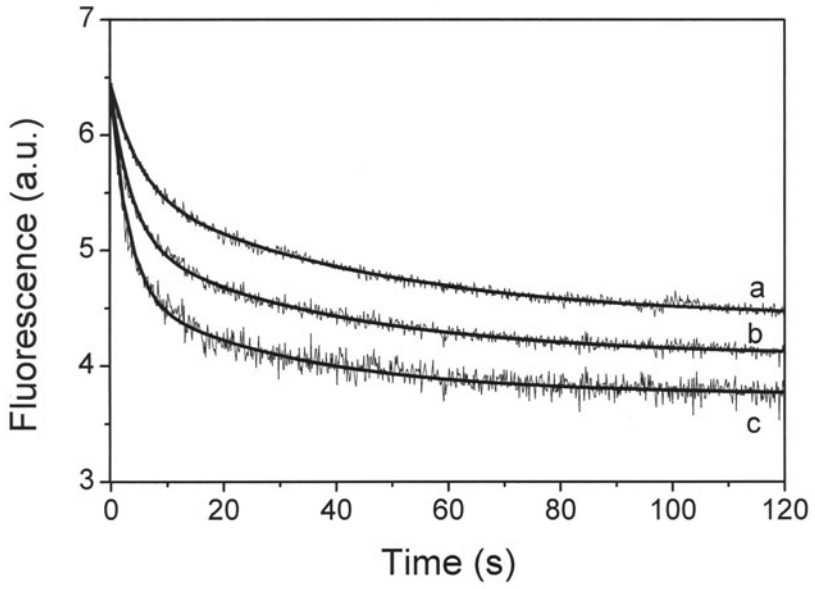

Figure 2. Changes in fluorescence intensity of pyrene-labelled F-actin during its interaction with calponin at various ratios of both proteins

Curves a, b and c correspond to 0.8, 1.2 and 1.6 μM calponin, respectively, mixed with 2 μM F-actin in solutions described in Fig. 1. Bold lines are double exponential fits. Corresponding rate constants and amplitudes are given in Table 1. Measurements were performed at 20°C

a significant change in light scattering signal (Fig.1C) without an appreciable change in fluorescence argues that the quenching in fluorescence observed in Fig. 2 (curve c) is not an artefact arising from the increasing turbidity producing an inner filter effect but reflects an environmental change in the pyrene labelled actin residue.

DISCUSSION

Combination of the light scattering and fluorescence methods to analyse the kinetics of the calponin–actin interaction allowed us to investigate both the binding of calponin to actin filaments and structural changes in the actin filaments accompanying this process. The results revealed that the mode of calponin binding to actin filaments depends on the ratio of both proteins: at a low ratio, elongated calponin molecules bind longitudinally along the filaments, at high a ratio transverse binding predominates facilitating bundling of actin filaments. At intermediate ratios, calponin molecules initially bind in a random fashion and subsequently rearrange into the longitudinal position. The molecules, which preserve the transverse orientation, form bundles of actin filaments. These results confirm our earlier proposition (Kołakowski *et al.*, 1997) of a two-site model of calponin-F-actin interaction.

The profiles of the fluorescence intensity changes were similar and independent of the calponin to actin ratio, thus, the mode of calponin binding to actin filaments. The detailed kinetic analysis showed that the fluorescence quenching process consists of two components similar in amplitude and different in rate. This is in agreement with the observation of Mino *et al.* (1998) that the peptides covering the two distinct actin-binding sites of calponin reduce the fluorescence intensity of pyrene-labelled actin to the same extent. The rate of fluorescence decrease was lower than the rate of binding of calponin to F-actin, which indicates that fluorescence quenching of pyrene-conjugated to Cys-374 of actin is produced by an intrinsic conformational change in the actin filaments induced by calponin binding and not by calponin binding *per se*. This is consistent with earlier observations of Noda *et al.* (1992) that, upon treatment with calponin, the spectra of filamentous actin became similar to those of monomeric actin pointing to a reversal of actin monomer conformation in filaments towards that of a free state. Lack of correlation between the rate of fluorescence changes and the rate of the light scattering increase resulting from bundling of actin filaments by calponin excluded the participation of actin filament bundling in inducing those structural alterations. A similar result was previously reported by Tang *et al.* (1997).

The different mode of calponin binding to actin, proposed above, seems to be in agreement with the data of Bartegi *et al.* (1999) on the interaction of the C-terminal domain of calponin with F-actin. The authors observed, in the presence of an excess of labelled F-actin (at Cys-374) over acrylodan-labelled calponin (at Cys-273), significant changes in the intensity and maximum emission wavelength of fluorescence, which indicates a close proximity between the labelled amino-acid residues of both proteins. At the molar ratio of calponin to actin of 1:1.2, the distance measured between the labels increased to 2.9 nm.

The physicochemical methods applied so far in studies of the calponin–F-actin interaction, i.e., fluorescence intensity of pyrene-labelled actin (Mino *et al.*, 1998; Noda *et al.*, 1992; and this work), polarized fluorescence (Borovikov *et al.*, 1996) and light scattering (this work) indicate that calponin induces changes in the shape and flexibility of actin filaments due to conformational alterations in actin monomers. Direct evidence for such alterations was obtained by subtracting the densities associated with pure F-actin from those for the F-actin–calponin complexes obtained from the electron micrographs of neg-

atively stained filaments (Hodgkinson *et al.*, 1997).

In conclusion, we suggest that the physiological significance of calponin to actin binding consist in that:

- the changes induced by calponin in actin filament structure, slows down the crossbridge cycle and thus inhibit actomyosin ATPase activity,
- independent of the above changes, fast bundling of actin filaments by a larger amount of calponin takes part in the formation of the actin cytoskeleton and its remodelling during the contraction-relaxation cycle of smooth muscle.

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REFERENCES

Barshop BA, Wren RF, Frieden C. (1983) Analysis of numerical methods for computer simulation of kinetic processes: development of KINSIM-a flexible, portable system. *Anal Biochem.*; **130**: 134–45.

Bartegi A, Roustan C, Kassab R, Fattoum A. (1999) Fluorescence studies of the carboxyl-terminal domain of smooth muscle calponin effects of F-actin and salts. *Eur J Biochem.*; **262**: 335–41.

Borovikov YuS, Horiuchi KY, Avrova SV, Chacko S. (1996) Modulation of actin conformation and inhibition of actin filament velocity by calponin. *Biochemistry.*; **35**: 13849–57.

Cantor CR, Schimmel PR. (1980) Biophysical chemistry. Part III. In *The behaviour of biological macromolecules*. Freeman WH, Company, pp 887–937, San Francisco.

Cooper JA, Walker SB, Pollard TP. (1983) Pyrene actin: documentation of the validity of a sensitive assay for actin polymerization. *J Muscle Res Cell Motil.;* **4**: 253–62.

El-Mezgueldi M, Marston SB. (1996) The effect of smooth muscle calponin on the strong and weak myosin-binding sites of F-actin. *J Biol Chem.*; **271**: 28161–7.

El-Mezgueldi M, Fattoum A, Derancourt J, Kassab R. (1992) Mapping of the functional domains in the amino-terminal region of calponin. *J Biol Chem.*; **267**: 15943–51.

El-Mezgueldi M, Mendre C, Calas B, Kassab R, Fattoum A. (1995) Characterization of the regulatory domain of gizzard calponin. Interactions of the 145–163 region with F-actin, calcium-binding proteins, and tropomyosin. *J Biol Chem.;* **270**: 8867–76.

El-Mezgueldi M, Strasser P, Fattoum A, Gimona M. (1996) Expressing functional domains of mouse calponin: involvement of the region around alanine 145 in the actomyosin ATPase inhibitory activity of calponin. *Biochemistry.*; **35**: 3654–61.

Hodgkinson JL, El-Mezgueldi M, Craig R, Vibert P, Marston SB, Lehman W. (1997) 3-D image reconstruction of reconstituted smooth muscle thin filaments containing calponin: visualization of interactions between F-actin and calponin. *J Mol Biol.*; **273**:150–9.

Houk T, Ue K. (1974) The measurement of actin concentration in solution: a comparison of methods. *Anal Biochem.*; **62**: 66–74.

Itoh T, Suzuki A, Watanabe Y, Mino T, Naka M, Tanaka T. (1995) A calponin peptide enhances Ca^{2+} sensitivity of smooth muscle contraction without affecting myosin light chain phosphorylation. *J Biol Chem.*; **270**: 20400–3.

Kołakowski J, Karkucińska A, Dąbrowska R. (1997) Calponin inhibits actin-activated MgATPase of myosin subfragment 1 (S1) without displacing S1 from its binding site on actin. *Eur J Biochem.*; **243**: 624–9.

Kołakowski J, Makuch R, Stępkowski D, Dąbrowska R. (1995) Interaction of calponin with actin and its functional implications. *Biochem J.*; **306**: 199–204.

Kouyama T, Mihashi K. (1981) Fluorimetry study of *N*-(1-pyrenyl)iodoacetamide-labelled F-actin. Local structural change of actin protomer both on polymerization and on

binding of heavy meromyosin. *Eur J Biochem.*; **114**: 33–8.

Laemmli UK. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.*; **227**: 680–5.

Leinweber B, Tang JX, Stafford WF, Chalovich JM. (1999) Calponin interaction with alpha-actinin-actin: evidence for a structural role for calponin. *Biophys J.*; **77**: 3208–17.

Lu FW, Freedman MV, Chalovich JM. (1995) Characterization of calponin binding to actin. *Biochemistry.*; **34**: 11864–71.

Mabuchi KLiY, Tao T, Wang CL. (1996) Immunocytochemical localization of caldesmon and calponin in chicken gizzard smooth muscle. *J Muscle Res Cell Motil.*; **17**: 243–60.

Mino T, Yuasa U, Nakamura F, Naka M, Tanaka T. (1998) Two distinct actin-binding sites of smooth muscle calponin. *Eur J Biochem.*; **251**: 262–8.

Noda S, Ito M, Watanabe S, Takahashi K, Maruyama K. (1992) Conformational changes of actin induced by calponin. *Biochem Biophys Res Commun.*; **185**: 481–7.

North AJ, Gimona M, Cross RA, Small JV. (1994) Calponin is localised in both the contractile apparatus and the cytoskeleton of smooth muscle cells. *J Cell Sci.*; **107**: 437–44.

Spudich JA, Watt S. (1971) The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J. Biol. Chem.*; **246**: 4866–71.

Stafford WF, Mabuchi K, Takahashi K, Tao, T. (1995) Physical characterization of calponin. A circular dichroism, analytical ultracentrifuge, and electron microscopy study. *J Biol Chem.*; **270**: 10576–9.

Szymanski PT, Grabarek Z, Tao T. (1997) Correlation between calponin and myosin subfragment 1 binding to F-actin and ATPase inhibition. *Biochem J.*; **321**: 519–23.

Takahashi K, Hiwada K, Kokubu T. (1986) Isolation and characterization of a 34,000-dalton calmodulin- and F-actin-binding protein from chicken gizzard smooth muscle. *Biochem Biophys Res Commun.;* **141**: 20–6.

Tang JX, Szymanski PT, Janmey PA, Tao T. (1997) Electrostatic effects of smooth muscle calponin on actin assembly. *Eur J Biochem.*; **247**: 432–40.

Walsh MP, Carmichael JD, Kargacin, G J. (1993) Characterization and confocal imaging of calponin in gastrointestinal smooth muscle. *Am J Physiol.*; **265**: C1371–8.