Phosphorylation by casein kinase II affects the interaction of caldesmon with smooth muscle myosin and tropomyosin

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Smooth muscle caldesmon was phosphorylated by casein kinase II, and the effects of phosphorylation on the interaction of caldesmon and its chymotryptic peptides with myosin and tropomyosin were investigated. The N-terminal chymotryptic peptide of caldesmon of molecular mass 27 kDa interacted with myosin. Phosphorylation of Ser-73 catalysed by casein kinase II resulted in a 2-fold decrease in the affinity of the native caldesmon (or its 27 kDa N-terminal peptide) for smooth muscle myosin. At

low ionic strength, caldesmon and its N-terminal peptides of molecular masses 25 and 27 kDa were retarded on a column of immobilized tropomyosin. Phosphorylation of Ser-73 led to a 2–4-fold decrease in the affinity of caldesmon (or its N-terminal peptides) for tropomyosin. Thus phosphorylation of Ser-73 catalysed by casein kinase II affects the interaction of caldesmon with both smooth muscle myosin and tropomyosin.

INTRODUCTION

In the early 1980s a new actin- and calmodulin-binding protein, caldesmon, was discovered in smooth muscles and in some non-muscle cells (Sobue et al., 1981, 1988). Caldesmon is involved in the regulation of smooth muscle contraction (Marston and Smith, 1985; Chalovich, 1988) and non-muscle motility (Walker et al., 1989; Yamashiro et al., 1990; Yamakita et al., 1990). Isolated caldesmon inhibits actin-activated myosin-ATPase, and this inhibitory effect of caldesmon is reversed by calmodulin and other calcium-binding proteins (Marston and Smith, 1985; Chalovich, 1988; Skripnikova and Gusev, 1989; Marston and Redwood, 1991). Thus, in conjunction with certain calcium-binding proteins caldesmon regulates the interaction of actin with myosin.

In vitro, caldesmon is phosphorylated by Ca^{2+} /calmodulin-dependent protein kinase (Vorotnikov et al., 1988; Sutherland and Walsh, 1989; Ikebe and Reardon, 1990), by Ca^{2+} /phospholipid-dependent protein kinase (Umekawa and Hidaka, 1985; Vorotnikov et al., 1988; Tanaka et al., 1990), by p34 cdc-2 kinase (Yamashiro et al., 1990; Mak et al., 1991) and by casein kinase type II (Vorotnikov et al., 1988; Wawrzynow et al., 1991). Phosphorylation by Ca^{2+} /calmodulin-dependent protein kinase inhibits the interaction of caldesmon with myosin (Sutherland and Walsh, 1989), whereas phosphorylation by Ca^{2+} /phospholipid-dependent protein kinase and cdc-2 kinase affect the interaction of caldesmon with actin (Yamashiro et al., 1990; Tanaka et al., 1990; Mak et al., 1991). Moreover, caldesmon is also phosphorylated *in vivo*, and some physiologically active compounds may affect caldesmon phosphorylation (Adam et al., 1989, 1990). All of these data indicate that caldesmon undergoes phosphorylation under certain conditions, and this may affect the ability of caldesmon to regulate the interaction of actin with myosin. This paper is devoted to the investigation of caldesmon phosphorylation by casein kinase II, and we study the effect of phosphorylation on the interaction of caldesmon with myosin and tropomyosin.

MATERIALS AND METHODS

Protein purification

Caldesmon and tropomyosin were isolated by the method of Lynch and Bretscher (1986) from duck gizzard. Actin was purified from rabbit skeletal muscle according to Spudich and Watt (1971). Dephosphorylated myosin from chicken gizzard was isolated by a previously described method (Sellers et al., 1981) and was stored for less than 1 month in 50 % glycerol at -20°C . Before experiments, myosin was dialysed overnight against 100 vol. of 10 mM imidazole, pH 7.0, containing 1 mM MgATP, 25–140 mM NaCl and 10 mM mercaptoethanol. Casein kinase II was isolated from rat liver according to Matsumura and Takeda (1972).

Phosphorylation of caldesmon

Caldesmon (0.4–2.0 mg/ml) was added to buffer containing 8 mM Tris, 12 mM KH_2PO_4 , 5 mM MgCl_2 , 30 mM NaCl, 2 mM dithiothreitol (DTT) and 100–200 μM ATP (including $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$). The casein kinase concentration was less than 30 $\mu\text{g}/\text{ml}$. The extent of caldesmon phosphorylation, determined as described earlier (Vorotnikov et al., 1988), was equal to 0.1–0.6 mol of phosphate per mol (89 kDa) of caldesmon. The control experiment did not contain casein kinase II. After incubation, phosphorylated and non-phosphorylated caldesmon were dialysed two or three times against 100 vol. of 10 mM Pipes, pH 7.0, containing 0.1 mM DTT and 0.1 mM phenylmethanesulphonyl fluoride (PMSF). In some experiments both phosphorylated and dephosphorylated caldesmon were subjected to chymotrypsinolysis. Caldesmon in the buffer used for phosphorylation was mixed with chymotrypsin (caldesmon/chymotrypsin ratio 1000:1, w/w) and incubated for 5–10 min at 30°C . The reaction was stopped by the addition of PMSF to a final concentration of 1–2 mM, and the peptides thus obtained were dialysed two or three times against 100 vol. of 10 mM Pipes, pH 7.0, containing 0.1 mM DTT and 0.1 mM PMSF.

Interaction of caldesmon and its peptides with myosin

Interactions were investigated by ultracentrifugation (Velaz et al., 1990). Caldesmon or its chymotryptic peptides (1.5–2.0 mg/ml) were centrifuged at 105000 *g* for 30 min at 20 °C and the pellet was discarded. The supernatant was mixed with smooth muscle myosin (final concentration of myosin, 0.4–0.8 mg/ml; final concentration of caldesmon or its peptides, 1.1–1.4 mg/ml) in 10 mM imidazole, pH 7.0, containing 1 mM MgATP, 25–140 mM NaCl and 10 mM mercaptoethanol. After incubation for 20 min at room temperature, the samples were centrifuged at 105000 *g* for 30 min. The pellet and supernatant were analysed by SDS/PAGE (Laemmli, 1970). After routine staining and destaining procedures the gels were subjected to autoradiography, and both gels and autoradiograms were scanned on an Ultrosan XL (LKB) densitometer. There was a linear relationship between the quantity of caldesmon peptides loaded on the gel and the area under the corresponding peaks on the densitogram and autoradiogram.

The relative binding of phosphorylated (CD_p) and unphosphorylated (CD) caldesmon to myosin was assessed by the following approach. We determined the areas of the Coomassie Blue R-250-stained bands of caldesmon (or caldesmon peptides) and the areas of the corresponding bands on the autoradiograms. Using these values and knowing the stoichiometry of phosphorylation, the CD_p/CD ratios in the supernatant and in the pellet were calculated. Assuming equimolar complex formation and taking into account that:

$$K_p = \frac{[M-CD_p]}{[M] \cdot [CD_p]} \text{ and } K = \frac{[M-CD]}{[M] \cdot [CD]}$$

where K and K_p are association constants for unphosphorylated and phosphorylated caldesmons with myosin, $[M-CD]$ and $[M-CD_p]$ are the concentrations of myosin–caldesmon complexes, and $[CD]$, $[CD_p]$ and $[M]$ are the concentrations of free caldesmons and myosin which are not involved in complex formation, we may conclude that:

$$\frac{K_p}{K} = \frac{[M-CD_p] \cdot [M] \cdot [CD]}{[M] \cdot [CD_p] \cdot [M-CD]}$$

The ratio $[M-CD_p]/[M-CD]$ is equal to $[CD_p]/[CD]$ in the pellet, whereas $[CD_p]/[CD]$ is equal to $[CD_p]/[CD]$ in the supernatant. Thus:

$$\frac{K_p}{K} = \frac{([CD_p]/[CD])_{\text{pellet}}}{([CD_p]/[CD])_{\text{supernatant}}}$$

Thus, by calculating the ratio $[CD_p]/[CD]$ in the supernatant and in the pellet, we can determine the ratio of association constants for phosphorylated and unphosphorylated caldesmons.

Caldesmon–tropomyosin interactions

These were investigated by three independent methods. Caldesmon or its chymotryptic peptides were added to a mixture of rabbit skeletal F-actin and smooth muscle tropomyosin in 10 mM imidazole, pH 7.0, containing 2 mM $MgCl_2$ and 1 mM mercaptoethanol. The final concentrations of caldesmon (or its peptides) varied from 0.06 to 0.3 mg/ml; the concentrations of F-actin and tropomyosin were 0.14 and 0.07 mg/ml respectively. After incubation for 30 min at room temperature, the samples were subjected to ultracentrifugation at 105000 *g* for 30 min. The

protein composition of both pellet and supernatant was analysed by SDS/PAGE (Laemmli, 1970).

In the second method, smooth muscle tropomyosin was immobilized on CNBr-activated Sepharose as recommended by Pharmacia. The column (1.2 cm $\times$ 3 cm) was equilibrated with buffer A (10 mM Pipes, pH 7.0, 0.1 mM DTT and 0.1 mM PMSF). Intact caldesmon or a mixture of caldesmon peptides (0.7–1.2 mg) having different extents of phosphorylation (0.1–0.6 mol of phosphate/mol of protein) was dialysed two or three times against 100 vol. of buffer A and loaded on to the column of immobilized tropomyosin. After loading of the sample (200–800 μ l) the column was washed with 3–8 bed volumes of buffer A and eluted with buffer A containing 0.25 M NaCl. The protein concentration (Spector, 1978) and the radioactivity, estimated by the Čerenkov method, were determined in each fraction (1–1.2 ml) and in the sample loaded on the column.

The third approach for the investigation of caldesmon–tropomyosin interactions consisted of the titration with caldesmon of tropomyosin modified by 5-dimethylaminonaphthalene-1-sulphonyl (Dns) chloride. The procedure for tropomyosin modification was similar to that described by Burtnick and Bhangu (1986). Tropomyosin in 40 mM potassium borate, pH 8.0, 0.5 M KCl and 1 mM DTT was mixed with a 100-molar excess of Dns chloride and incubated for 3 h at room temperature. The excess of Dns chloride was removed by five rounds of dialysis against 100 vol. of 25 mM Tris/HCl, pH 7.0, 0.3 M KCl and 1 mM DTT. The extent of modification was determined by assuming $\epsilon_{340} = 4.3 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for the Dns moiety and $\epsilon_{277} = 2.17 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for tropomyosin. Under the conditions described, the extent of modification was equal to 0.3 mol of Dns/mol of tropomyosin. The fluorescence was measured with a Hitachi F-3000 fluorimeter in buffer containing 10 mM Tris/HCl, pH 7.5, and 0.1 mM DTT. The final concentration of Dns-tropomyosin was 0.5 μ M. Fluorescence was excited at 365 nm and was recorded at 505 nm. After correction for dilution and scattering, the dependence of the fluorescence on the quantity of added caldesmon was plotted. Binding curve analyses were carried out assuming either non-cooperative (Watson et al., 1990a) or a co-operative interaction of tropomyosin with caldesmon. The calculations performed by both methods gave similar results.

RESULTS

Interaction of caldesmon with myosin

Ikebe and Reardon (1988) found that caldesmon is able to interact with smooth muscle myosin. Further investigations (Velaz et al., 1990) have shown that the first 170 residues of caldesmon are directly involved in this interaction. Recently published data (Wawrzynow et al., 1991) indicate that casein kinase II predominantly phosphorylates sites located in the N-terminal part of caldesmon. Under the conditions used more than 60% of phosphate was bound to Ser-73, whereas phosphorylation of any other site did not exceed 6–8%. Since the main site phosphorylated by casein kinase II is located in the peptide section involved in the interaction with myosin, it was reasonable to investigate the effect of phosphorylation on myosin–caldesmon interactions.

By subjecting a mixture of myosin and caldesmon to ultracentrifugation under conditions where the molar concentration of caldesmon was 6–12 times higher than that of myosin and determining the ratio $[CD_p]/[CD]$ in both the supernatant and the pellet, we found that phosphorylation by casein kinase II leads to an approx. 2-fold decrease in the apparent affinity

Table 1 Effect of phosphorylation on the interaction of intact caldesmon and its N-terminal 27 kDa peptide with unphosphorylated smooth muscle myosin

Conditions of ultracentrifugation are described in the Materials and methods section. K/K_p denotes the ratio of association constants (with myosin) for unphosphorylated and phosphorylated protein.

Protein	Ionic strength (mM NaCl)	K/K_p
Intact caldesmon	60	1.85 ± 0.18 ($n = 10$)
27 kDa N-terminal peptide	70	1.84 ± 0.18 ($n = 10$)
	110	2.51 ± 0.35 ($n = 4$)

Figure 1 Interaction of intact caldesmon and its chymotryptic peptides with smooth muscle myosin

Native caldesmon (a) or a mixture of its chymotryptic peptides (b), previously phosphorylated with casein kinase II, was mixed with smooth muscle myosin and subjected to ultracentrifugation. The protein composition of the initial mixture (i), supernatant (s) and pellet (p) after ultracentrifugation was analysed by SDS/PAGE. A, Coomassie Blue R-250-stained gels; B, autoradiogram.

constant for the interaction between caldesmon and myosin (Table 1).

Phosphorylated caldesmon was cleaved by chymotrypsin, and the interaction of the generated caldesmon peptides with myosin was investigated by ultracentrifugation (Figure 1). In good agreement with our earlier published data (Vorotnikov et al.,

Figure 2 Effect of ionic strength on the interaction of chymotryptic peptides of caldesmon with smooth muscle myosin

Isolated myosin (1.5 μ M) (1) or myosin in the presence of caldesmon peptides (12.0 μ M) (2–5) was ultracentrifuged and supernatant (s) and pellet (p) were subjected to SDS/PAGE. The concentration of NaCl in the incubation mixture was 25 (1 and 2), 50 (3), 70 (4) and 110 mM (5) respectively.

1988) intact caldesmon (molecular mass 140 kDa) and its chymotryptic peptides of 110, 27 and 25 kDa contain the sites of phosphorylation. The 110 kDa peptide is a large fragment of caldesmon retaining its N-terminal part. This peptide interacts with and is co-sedimented with myosin. The two short peptides of 25 and 27 kDa are also N-terminal fragments of caldesmon. Both of these peptides were co-sedimented with myosin at low ionic strength, whereas predominantly the 27 kDa peptide was co-sedimented with myosin at an ionic strength greater than 70 mM NaCl (Figure 2). On varying the extent of phosphorylation and determining the ratio of phosphorylated to non-phosphorylated 27 kDa peptide in the supernatant and the pellet, we found that the apparent association constant of the phosphorylated 27 kDa peptide was roughly 2-fold lower than the corresponding value for the unphosphorylated peptide (see Figure 1 and Table 1). The effect of phosphorylation was slightly increased at high ionic strength, reaching 2.5-fold at 110 mM NaCl. Thus phosphorylation of Ser-73 inhibits the interaction of caldesmon with smooth muscle myosin.

Interaction of caldesmon with tropomyosin

A number of studies have indicated that, at low ionic strength, caldesmon interacts with tropomyosin (Smith et al., 1987; Fujii et al., 1988; Hayashi et al., 1989; Katayama et al., 1989; Watson et al., 1990a,b). It is generally accepted that the tropomyosin-binding site is located in the C-terminal 35 kDa fragment of caldesmon (Fujii et al., 1988; Hayashi et al., 1989). However, the data of Katayama et al. (1989), Moody et al. (1990) and Watson et al. (1990b) indicate that there is a second tropomyosin-binding site, located in the N-terminal region of caldesmon.

To check this assumption, we performed two types of experiments. In the first, a mixture of F-actin, tropomyosin and chymotryptic peptides of caldesmon was subjected to ultracentrifugation. At low ionic strength the pellet thus obtained contained chymotryptic peptides of caldesmon of 40 kDa and 19–21 kDa, derived from the C-terminal part of caldesmon, as well as the 25 and 27 kDa peptides from the N-terminal region. The 40 and 19–21 kDa peptides are known to interact with both actin and tropomyosin (Szpacenko and Dabrowska, 1986; Hayashi et al., 1989; Wang et al., 1991), and therefore they are expected to be

Figure 3 Interaction of chymotryptic peptides of caldesmon with immobilized tropomyosin

The mixture of chymotryptic peptides of caldesmon, phosphorylated by casein kinase II, was loaded on the affinity column, and after washing, the peptides retarded on the column were eluted with 0.25 M NaCl (marked by the arrow). The protein composition of the mixture loaded on the column (1), of fraction 6 (2) and of fraction 33 (3) was analysed by SDS/PAGE (A) and autoradiography (B). The scale of apparent molecular masses is given on the right. The extent of caldesmon phosphorylation was equal to 0.11 (●) and 0.32 (○) mol of phosphate/mol of protein.

co-sedimented with the actin–tropomyosin complex. Detection of 25 and 27 kDa peptides in the pellet may indicate that, at low ionic strength, these peptides interact with either tropomyosin or the C-terminal peptides of caldesmon that are already bound to actin–tropomyosin complex.

The second approach consisted of the application of affinity chromatography for determination of the interaction of caldesmon peptides with tropomyosin. In this case we loaded a mixture of caldesmon chymotryptic peptides on to a column of immobilized tropomyosin and analysed the protein composition of the fractions that were retarded on the column. Fraction 33, i.e. the peak fraction eluted with 0.25 M NaCl, was enriched in peptides of 25 and 27 kDa which contained the sites phosphorylated by casein kinase II (Figure 3). A large 100 kDa peptide containing the phosphorylation site also interacted with immobilized tropomyosin. The quantity of peptides interacting with tropomyosin was decreased if the ionic strength of the starting buffer was increased up to 50 mM. Although caldesmon peptides were bound to the tropomyosin–Sepharose only at low ionic strength, this interaction seemed to be rather specific, as N-terminal peptides of both caldesmon and tropomyosin are negatively charged at pH 7.0.

To investigate the effects of phosphorylation, we loaded on to the column of immobilized tropomyosin a mixture of chymotryptic peptides of caldesmon that were phosphorylated to different extents. An increase in the extent of phosphorylation resulted in a decrease in the quantity of peptides retarded on the column (Figure 3). A similar effect was observed in the case of native caldesmon. The specific radioactivity of caldesmon or its peptides loaded on to the column was 3–4 times higher than that in the fractions retarded by tropomyosin–Sepharose. Thus phosphorylation of Ser-73 inhibits the interaction of both intact caldesmon and its N-terminal peptides with tropomyosin.

To verify this assumption, we investigated the effects of phosphorylated and unphosphorylated caldesmon on the fluorescence of tropomyosin labelled by Dns chloride. Titration of Dns-tropomyosin with caldesmon leads to 1.4–1.6-fold increase in the fluorescence intensity, and to a blue shift of the fluorescence maximum. All of these changes were observed only at low ionic

Figure 4 Titration curve of Dns-tropomyosin (0.45 μ M) with unphosphorylated (curve 1) and phosphorylated (0.53 mol of phosphate/mol of protein) (curve 2) caldesmon

strength. An increase in the ionic strength to 50 mM abolished the changes in Dns-tropomyosin fluorescence induced by caldesmon. This is in a good agreement with the data of Watson et al. (1990a) who found that, at high ionic strength (greater than 50 mM), caldesmon was unable to affect the fluorescence of fluorescently labelled tropomyosin. As shown in Figure 4, both phosphorylated and non-phosphorylated caldesmon increased the fluorescence of Dns-tropomyosin; however, a higher concentration of phosphorylated caldesmon was needed to give a half-maximal increase in fluorescence. Phosphorylation of caldesmon only slightly affected the maximal intensity of fluorescence ($F_{\max}$) and the stoichiometry of the caldesmon–tropomyosin interaction. At the same time phosphorylation decreased the association constant from $(2.7 \pm 1.3) \times 10^6 \text{ M}^{-1}$ ($n = 6$) for unphosphorylated caldesmon to $(1.3 \pm 0.6) \times 10^6 \text{ M}^{-1}$ ($n = 6$) for

partly phosphorylated caldesmon (0.4–0.6 mol of phosphate/mol of protein). Thus, by using two independent methods we have shown that phosphorylation of caldesmon inhibits its interaction with tropomyosin.

DISCUSSION

The data presented indicate that phosphorylation of residue Ser-73 of caldesmon decreases the interaction of the protein with myosin. A similar effect was described by Sutherland and Walsh (1989), who showed that phosphorylation of caldesmon by so-called caldesmon kinase prevented its interaction with myosin. Caldesmon kinase was subsequently identified as Ca^{2+} /calmodulin-dependent protein kinase (Scott-Woo et al., 1990). This enzyme preferentially phosphorylates Ser-73, Ser-26, Ser-726 and Ser-587 of smooth muscle caldesmon (Ikebe and Reardon, 1990), i.e. sites located in both the short N-terminal (23.5 kDa) and the C-terminal (21.2 kDa) cyanolytic peptides of caldesmon (Sutherland and Walsh, 1989). Therefore it was difficult to determine which sites needed to be phosphorylated in order to affect the interaction of caldesmon with myosin. Casein kinase II predominantly phosphorylates Ser-73 of smooth muscle caldesmon (Wawrzynow et al., 1991), and phosphorylation of this residue significantly decreased the interaction of caldesmon with myosin. Summing up, we conclude that phosphorylation of Ser-73 and/or Ser-26 of caldesmon may affect its interaction with myosin.

It is well known that caldesmon interacts with tropomyosin (Fujii et al., 1988; Hayashi et al., 1989; Katayama et al., 1989; Watson et al., 1990a,b). The C-terminal peptide of caldesmon of molecular mass 35–38 kDa is able to interact with immobilized tropomyosin (Fujii et al., 1988; Hayashi et al., 1989). Experiments using several deletion mutants of caldesmon also demonstrated that the tropomyosin-binding site is located in the C-terminal part of caldesmon (Hayashi et al., 1991). However, under certain conditions other regions of caldesmon are able to interact with immobilized tropomyosin. The short N-terminal peptide of 28 kDa (Hayashi et al., 1989) and larger peptides of 80 and 110 kDa (Katayama et al., 1989) are also retarded on the immobilized tropomyosin. The stoichiometry of the caldesmon–tropomyosin complex varied from 0.5 to 2.0 (Watson et al., 1990b), indicating that the caldesmon molecule is able to interact with more than one molecule of tropomyosin. Electron microscopy indicated that, in the absence of tropomyosin, the elongated caldesmon molecule is fixed on the actin filament by only one (probably C-terminal) end, whereas the second (N-terminal) part retains its freedom and does not interact with actin (Moody et al., 1990). In the presence of tropomyosin both ends of caldesmon are fixed on the thin filament. A deletion mutant of caldesmon missing the C-terminal portion was also able to interact with pyrene-labelled tropomyosin (Redwood et al., 1990). All of these data suggest that caldesmon contains two tropomyosin-binding sites. One of these is located in the C-terminal part and the other in the N-terminal part of caldesmon. In our experiments, short chymotryptic peptides of caldesmon, of molecular mass 25 and 27 kDa, containing the sites phosphorylated by casein kinase II, interact with immobilized tropomyosin and are co-sedimented with the actin–tropomyosin complex. Although unequivocal evidence of the presence of a tropomyosin-binding site in the N-terminal part of caldesmon requires additional experiments with isolated 25 or 27 kDa peptides, the data presented indicate that caldesmon indeed contains a second tropomyosin-binding site. Phosphorylation of Ser-73 diminishes the interaction of caldesmon with tropomyosin,

as is shown by both affinity chromatography and fluorescence spectroscopy.

The question arises as to how the same N-terminal peptide (residues 1–170) of caldesmon interacts with both myosin and tropomyosin. It was suggested that caldesmon interacts with the S-2 region of smooth muscle myosin (Ikebe and Reardon, 1988; Hemric and Chalovich, 1990). This portion of myosin contains a characteristic 7-residue repeat pattern with a periodic distribution of positively and negatively charged amino acid residues (Yanagisawa et al., 1987). The same is true for the smooth muscle tropomyosin sequence (Sanders and Smillie, 1985). The N-terminal part of caldesmon contains two stretches (residues 2–39 and 76–113) which tend to form α -helices (Bryan et al., 1989; Marston and Redwood, 1991) consisting of four or five heptades with a periodic distribution of negatively and positively charged residues. For example, negatively charged residues are located at positions 2, 9, 16, 23 and 31 of the first α -helix and 77, 84, 91, 98, 106 and 113 of the second α -helix, whereas positively charged residues are located in positions 6, 12 and 19 of the first helix and 81, 96, 103 and 110 of the second helix. A periodic distribution of the charged residues was previously found in the tropomyosin-binding site of troponin T (Leszyk et al., 1987), and it was supposed that these structures may be involved in the formation of triple-stranded coiled-coil (Nagano et al., 1982). The data presented here suggest that the periodic distribution of the charged residues in the N-terminal region of caldesmon may allow the interaction of this protein with the coiled-coil structures of the S-2 portions of myosin and tropomyosin.

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