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Ca^{2+}-dependent regulation of vascular smooth-muscle caldesmon by S.100 and related smooth-muscle proteins

Kevin PRITCHARD and Steven B. MARSTON
Cardiac Medicine Department, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, U.K.

1. We have investigated the ability of bovine brain S.100, and of three related proteins from sheep aorta smooth muscle, to confer Ca^{2+}-sensitivity on thin filaments reconstituted from smooth-muscle actin, tropomyosin and caldesmon. 2. At 37 °C in pH 7.0 buffer containing 120 mM-KCl, approximately stoichiometric amounts of S.100 reversed caldesmon's inhibition of the activation of myosin MgATPase by smooth-muscle actin–tropomyosin. The [S.100] which reversed by 50% the inhibition by caldesmon (the E.C.50) was 2.5 μM when [caldesmon] = 2–3 μM in the assay mixture. When [KCl] was decreased to 70 mM, E.C.50 = 11.5 μM; at 25 °C in 70 mM-KCl, up to 20 μM-S.100 had no effect. When skeletal-muscle actin rather than smooth-muscle actin was used to reconstitute thin filaments, 20 μM-S.100 did reverse inhibition by caldesmon, at 25 °C in buffer containing 70 mM-KCl. This dependence on conditions is also characteristic of the calmodulin–caldesmon interaction. 3. These results suggested that S.100 or a related protein might interact with caldesmon in smooth muscle. We therefore attempted to prepare such a protein from sheep aorta. Three proteins were purified: an M_r-17000 protein (yield 16 mg/kg), an abundant M_r-11000 protein (yield 48 mg/kg), and an M_r-9000 protein (yield 4 mg/kg). Neither of the last two low-M_r proteins had any effect on activation of myosin MgATPase by reconstituted thin filaments. The protein of M_r 17000 had Ca^{2+}-sensitizing activity, and behaved exactly like brain calmodulin in the assay system.

INTRODUCTION

The contraction of smooth muscle is controlled by the intracellular concentration of free Ca^{2+}. The actomyosin contractile apparatus of smooth muscle is regulated by Ca^{2+} [1,2], which binds to regulatory proteins; distinct systems control the actin-based thin filaments and the thick (myosin) filaments. The Ca^{2+}-dependent regulatory component of the well-studied myosin-linked system is known to be calmodulin [3]. However, the Ca^{2+}-binding component of the thin filaments has never been identified. In addition to actin and tropomyosin, 'native' Ca^{2+}-regulated thin filaments [4], isolated intact from smooth muscles, contain the regulatory protein caldesmon [5,6], at a ratio of 1 molecule of caldesmon per 16 actin monomers [7]. Experiments on the reconstitution of purified smooth-muscle proteins into synthetic thin filaments have shown that caldesmon binds to actin–tropomyosin and inhibits its activation of myosin MgATPase [8,9]. The mechanism of regulation in intact native thin filaments likewise depends on caldesmon [10], and has been demonstrated with native thin filaments from vascular, visceral and uterine smooth muscle [6]. This inhibition is not Ca^{2+}-sensitive, but can be rendered Ca^{2+}-dependent by a suitable Ca^{2+}-binding regulatory protein which can bind to caldesmon, reversing its inhibition of actin–tropomyosin. Caldesmon was originally isolated as a calmodulin-binding protein [5], and a Ca^{2+}-sensitive thin-filament system can be reconstituted by using brain calmodulin [8,11,12].

However, it is by no means certain that native thin filaments are regulated by calmodulin; the calmodulin-regulated system is a poor mimic of the native Ca^{2+}-sensitive thin filaments [13–15]. The binding of caldesmon to actin–tropomyosin has been shown to be the same in native thin filaments as in the reconstituted system [7], and the discrepancies between the native and synthetic systems must be ascribed to the calmodulin. A large excess of calmodulin over caldesmon is required, except at 37 °C in buffers containing 120 mM-KCl [9], whereas the native thin filaments are more Ca^{2+}-sensitive at lower temperatures [7], and do not contain the large amounts of calmodulin which would be required. The Ca^{2+}-sensitizing factor of the native filaments is retained after washes in EGTA, yet calmodulin only binds to caldesmon in the presence of Ca^{2+} [16]. Furthermore, crude Ca^{2+}-sensitizing preparations from aorta can regulate MgATPase in the reconstituted system under conditions (25 °C and 70 mM-KCl-containing buffer) where calmodulin cannot [16]: this Ca^{2+}-sensitizing activity is rapidly destroyed at 80 °C [15], whereas the calmodulin–caldesmon interaction is very heat-stable [17]. However, reconstitution of Ca^{2+}-sensitive thin filaments has never been demonstrated with a pure protein from smooth muscle. We have therefore investigated the properties of Ca^{2+}-binding proteins other than calmodulin, in order to identify the intrinsic Ca^{2+}-dependent regulator of the thin filaments.

S.100 proteins belong to the 'E–F hand' group of Ca^{2+}-binding proteins and are found in various types of mammalian cell as homo- and hetero-dimers of α and β subunits [18,19]. S.100 proteins have been reported to regulate diverse cellular processes by binding to target proteins in a Ca^{2+}-dependent manner [20,21]. Recently brain S.100 protein has been shown to bind to caldesmon and to reverse caldesmon inhibition [22,23], and it has been suggested that S.100 may be the physiological regulator of caldesmon [22].

We have examined the regulation of reconstituted thin filaments by S.100 from brain, and have investigated whether smooth muscle contains substantial amounts of S.100 or any similar protein, which could be responsible for thin-filament-linked regulation of actomyosin.

METHODS

Preparation of Ca^{2+}-binding proteins

Presumptive Ca^{2+}-binding proteins were obtained from tissue extracts of bovine brain or sheep aorta by means of phenyl-Sepharose chromatography [24]. Four separate preparations of S.100, each from about 500 g of bovine brain, were made by the method of Masure and co-workers [25]. The 2.5 cm × 20 cm column of phenyl-Sepharose (Pharmacia/LKB) was finally eluted with a series of Ca^{2+}/EGTA buffer mixtures [25], which progressively decreased the free [Ca^{2+}] on the column. The peak containing an M_r-10000 protein was purified further by anion-

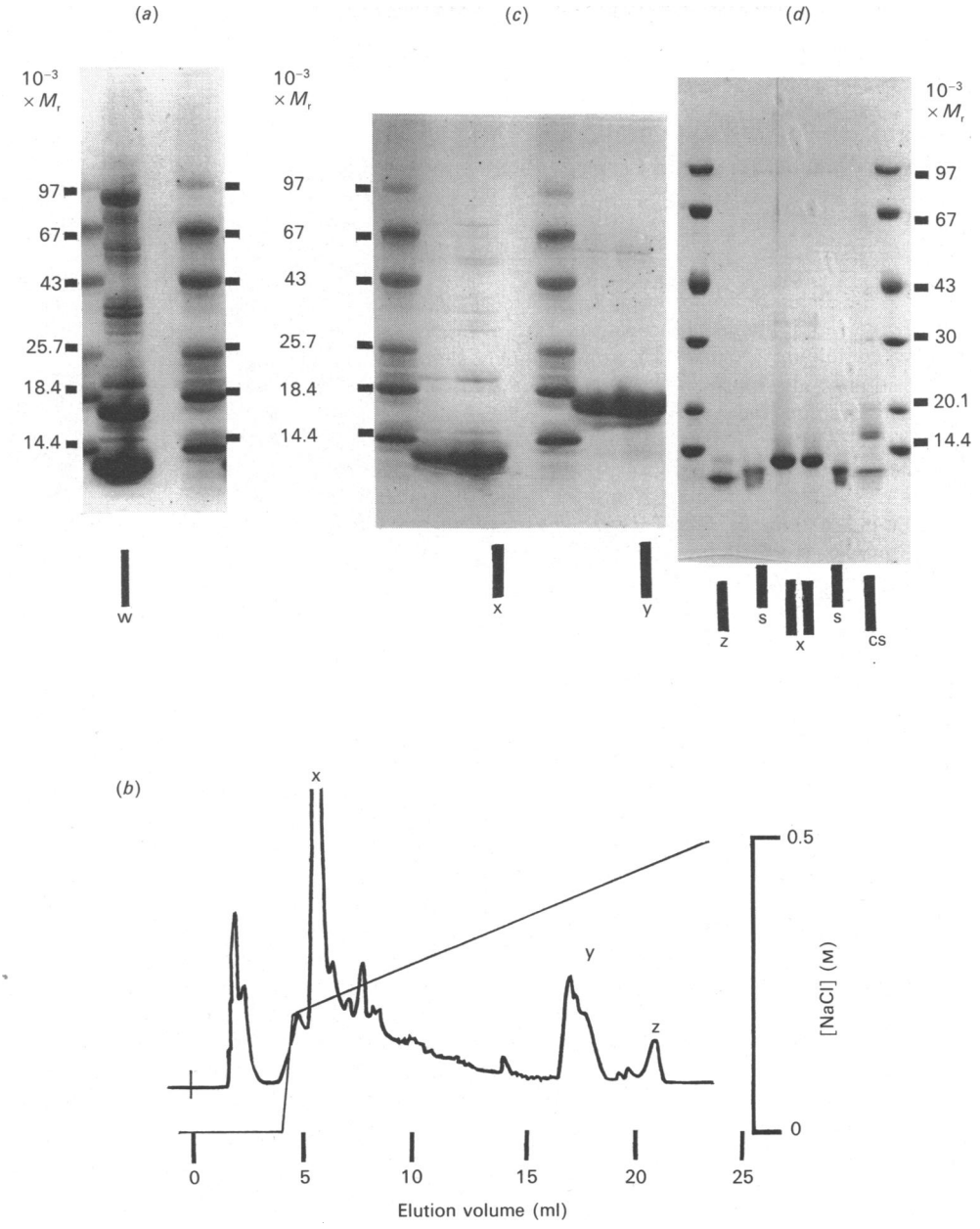

Fig. 1. Purification of S.100-like proteins from sheep aorta

Elution of phenyl-Sepharose with EDTA produced a complex mixture of proteins (panel *a*, lane w). Fractionation on Mono Q (panel *b*) produced three protein species, two of which, of M_r 11000 (panel *c*, lane x) and M_r 17000 (panel *c*, lane y), were obtained in high yield. Neither the protein of M_r 11000 (panel *d*, lane x) nor a band of M_r approx. 9000 (panel *d*, lane z) co-migrated with either brain S.100 (panel *d*, lane s) or brain calmodulin (panel *d*, lane cs; a mixture of calmodulin and S.100 from brain).

exchange chromatography on a 1 ml Mono-Q column, by using Pharmacia f.p.l.c. equipment. Samples were applied in 50 mM-Tris/HCl, pH 7.5, and eluted at 0.5 ml/min with a linear gradient of 0–0.5 M-NaCl in the buffer over 20 column volumes. For experiments with reconstituted thin filaments, mixed S.100 isoforms were used. The composition of one such preparation was determined by fractionating the mixture by a second stage of Mono-Q chromatography, using a 0–0.5 M-NaCl gradient as above run over 30 column volumes; S.100 isoforms were then eluted as separate peaks and were distinguished by mobility on non-SDS-containing gels. The proportion of each isoform was estimated by measuring the areas under the peaks of u.v. absorption.

A similar method was used in attempts to prepare S.100-like proteins from vascular smooth muscle. Sheep aortas (250 g) were homogenized in 650 ml of buffer (110 mM-KCl, 10 mM-NaCl, 3 mM-EDTA, 15 mM-2-mercaptoethanol, 10 mM-Mops, pH 7.2, containing as proteolysis inhibitors 2 μg/ml each of leupeptin, chymostatin and pepstatin and 0.1 mM-phenylmethanesulphonyl fluoride) and centrifuged at 23000 *g* for 15 min. The pellet was re-extracted and centrifuged as above, and the supernatants were combined and filtered through glass wool, before addition of $(NH_4)_2SO_4$ to 45% saturation, followed by centrifugation at 23000 *g* for 20 min. The 45%-satd-$(NH_4)_2SO_4$ supernatant was made 90% saturated with $(NH_4)_2SO_4$ and precipitated proteins were again collected by centrifugation; in one experiment, the mixture was acidified to pH 4.1 before centrifugation. This modification increased the yield of the M_r-11000 protein by

about 10-fold. The resulting protein pellet was resuspended in 500 ml of 50 mM-Tris/HCl, pH 8.0, containing enzyme inhibitors as above, then dialysed against column buffer (50 mM-Tris/HCl, pH 7.5, 10 mM-2-mercaptoethanol). The sample was clarified by centrifugation (45000 *g* for 1 h), 1 M-$CaCl_2$ stock was added to give 10 mM-$CaCl_2$, and the sample was applied at 15 ml/h to a 2.5 cm × 20 cm phenyl-Sepharose column. The column was washed with 500 ml of column buffer containing 0.1 mM-$CaCl_2$, then with 150 ml of column buffer containing 0.1 mM-$CaCl_2$ and 0.5 M-NaCl, and again with 150 ml of the buffer without NaCl. Because it was not known at what [Ca^{2+}] any putative Ca^{2+}-binding protein might be eluted from phenyl-Sepharose, [Ca^{2+}]-gradient elution was not attempted. The phenyl-Sepharose column was eluted with 5 mM-EDTA, and eluted proteins were precipitated by 90%-satd. $(NH_4)_2SO_4$. The resulting complex mixture was fractionated on Mono-Q anion exchanger as above. The composition of protein mixtures was determined by electrophoresis on 0.5 mm-thick 10 cm × 10 cm polyacrylamide gels (5–20% gradient) run at 400 V for 25 min (Cambridge Electrophoresis apparatus) in a buffer containing 1 g of SDS/l, 3 g of Tris base/l and 14.4 g glycine/l (pH 8.9). M_r values were determined from densitometric scans of 4–30% Pharmacia pre-cast gels. The gels were stained with Coomassie Blue. Protein concentrations were measured by the Lowry method. Molar concentrations of S.100, and of the smooth-muscle protein of M_r 11000, were calculated on the assumption that the proteins were dimers.

Preparation of contractile proteins

Vascular smooth-muscle proteins were prepared from sheep aorta. F-actin and caldesmon were prepared by fractionation of thin filaments by the method of Smith & Marston [8] or by applying the same fractionation procedure to heat-treated extract [26,27]. Tropomyosin was prepared by a modification [8] of the method of Bailey [28]. Myosin and F-actin were purified from rabbit skeletal muscle by standard procedures [29,30].

Reversal of caldesmon inhibition of actin-activated myosin MgATPase

Ca^{2+}-sensitizing activity was assayed as described by Pritchard & Marston [16]. Tubes contained 1–1.5 μM rabbit skeletal-muscle myosin, 24 μM actin (smooth or skeletal muscle) and 6 μM aorta tropomyosin, in 200 μl of 'ATPase' buffer containing 70 mM- or 120 mM-KCl, 5 mM-$MgCl_2$, 10 mM-NaN_3, 5 mM-K_2Pipes, pH 7.0 at assay temperature, 1 mM-dithiothreitol. The reaction was initiated by adding MgATP to 2 mM and terminated by addition of an 80 μl portion of the sample to 0.5 ml of 5% (w/v) trichloroacetic acid; the released P_i was assayed by colorimetry of a phosphomolybdate complex as described by Taussky & Schorr [31]. Each caldesmon preparation was titrated in a preliminary assay to determine the minimum concentration required for maximal ATPase inhibition (typically 1.5–2.4 μM, depending on assay conditions); this concentration of caldesmon was used to reconstitute thin filaments with potential Ca^{2+}-sensitizing factors.

RESULTS

Isoform composition of brain S.100

A peak eluted from phenyl-Sepharose at approx. pCa 5.5 was mainly composed of a protein of M_r 10000 by SDS/PAGE. Brain S.100 was purified from this peak by f.p.l.c. on a Mono-Q column at pH 7.5 (50 mM-Tris/HCl buffer). S.100 was eluted in a broad peak over NaCl concentrations between 270 and 340 mM. This mixed-isoform peak was used for experiments with reconstituted thin filaments. Subdivision of the S.100 peak into two pools, and a further stage of Mono-Q chromatography, allowed the isoforms to be obtained separately. The preparation tested contained S.100a_0 (α_2 homodimer), 4.5% (eluted between 270 and 280 mM-NaCl), S.100a (α–β heterodimer), 10.5% (280–300 mM-NaCl), and S.100b (β_2 homodimer), 85% (300–320 mM-NaCl). Calmodulin dissociated from phenyl-Sepharose at approx. pCa 6.0 and in subsequent Mono-Q chromatography was eluted between 300 and 380 mM-NaCl.

Purification of S.100-like proteins from aorta

Elution of the phenyl-Sepharose column with EDTA produced a mixture of proteins (Fig. 1*a*), from which three low-M_r proteins (Figs. 1*a*, 1*c* and 1*d*) were purified by anion-exchange on Mono-Q (Fig. 1*b*). An M_r-11500 (Fig. 1*c*, lane x) protein was eluted from Mono-Q between 170 and 180 mM-NaCl and was obtained in a yield of approx. 48 mg/kg wet wt. of aorta. An M_r-17000 (Fig. 1*c*, lane y) protein was eluted between 340 and 380 mM-NaCl, in a yield of approx. 16 mg/kg. A protein (Fig. 1*d*, lane z) of approx. M_r 9000 (yield approx. 4 mg/kg) was eluted between 400 and 440 mM-NaCl.

Reconstitution of Ca^{2+}-sensitive thin filaments with brain S.100

In the presence of 0.1 mM-$CaCl_2$, but not in 1 mM-EGTA, brain S.100 reversed the inhibition by caldesmon of the smooth-muscle actin–tropomyosin-activated myosin MgATPase (Fig. 2). Its ability to do so depended critically on experimental conditions (Fig. 2; Table 1). At 37 °C in buffer containing 120 mM-KCl, approximately stoichiometric amounts of S.100 reversed caldesmon inhibition, whereas as 37 °C in buffer containing 70 mM-KCl reversal of caldesmon inhibition required a considerable molar excess of S.100 over caldesmon (Table 1). S.100 at up to 20 μM appeared ineffective at 23 °C in buffer containing 70 mM-KCl. The same effect of conditions was observed with four independent preparations of S.100. When thin filaments were reconstituted by using skeletal-muscle actin with smooth-muscle caldesmon and tropomyosin, the Ca^{2+}-sensitivity conferred by

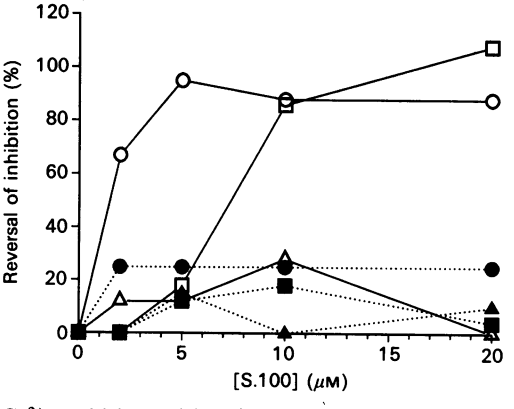

Fig. 2. Ca^{2+}-sensitizing activity of bovine brain S.100

In the presence of 0.1 mM-$CaCl_2$ (○, □, △), but not in the presence of 1 mM-EGTA (●, ■, ▲), bovine brain S.100 reversed the inhibition by 3.2 μM-caldesmon; the effect of S.100 clearly depended on experimental conditions. This pattern was seen with four preparations of S.100 and caldesmon. The MgATPase activity of 1.1 μM skeletal-muscle actin was activated by 24 μM aorta actin plus 6 μM aorta tropomyosin. '100%' is equivalent to an ATPase rate of 375 nmol of P_i/min per mg of myosin at 37 °C, 120 mM-KCl (○, ●), 907 nmol/min per mg at 37 °C, 70 mM-KCl (□, ■), and 228 nmol/min per mg at 25 °C, 70 mM-KCl (△, ▲). Myosin MgATPase rate remained linear during the incubation period (6 min at 37 °C; 10 min at 25 °C).

Table 1. Effect of bovine brain S.100 or calmodulin on inhibition of ATPase by caldesmon

E.C.50 is the concentration of Ca^{2+}-binding protein which, in the presence of 0.1 mM-$CaCl_2$, effected a 50% reversal of the activation by actin–tropomyosin of MgATPase activity of myosin. In the presence of 1 mM-EGTA, neither S.100 nor calmodulin had any effect. Molar concentrations of S.100 assume that the protein acts as a dimer of M_r 22000. Each preparation was tested at 5–7 concentrations over the range 0.5–20 μM. Assay conditions were essentially the same for S.100 or calmodulin. E.C.50 values for S.100 were estimated from the data sets for individual experiments, and are quoted as means ± S.D.; n is the number of independent protein preparations tested under each set of conditions. The data for calmodulin are included to facilitate comparison, and have been taken from Pritchard & Marston [16].

		S.100		Calmodulin	
Temperature (°C)	[KCl] (mM)	E.C.50 (μM)	n	E.C.50 (μM)	n
37	120	2.5 ± 1.1	4	4.0 ± 2.0	1
37	70	10.0 ± 5.8	3	14.0 ± 1.0	4
25	70	⩾ 100	4	⩾ 300	3

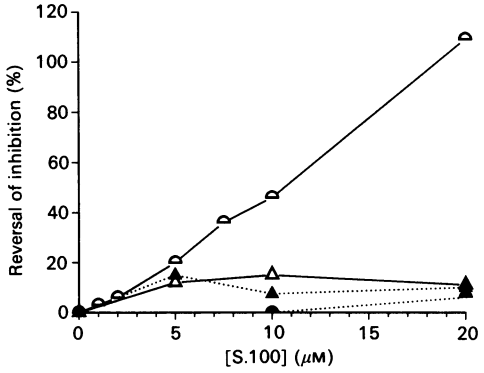

Fig. 3. Ca^{2+}-sensitizing activity of brain S.100 depends on actin isoform

In the presence of 0.1 mM-$CaCl_2$, brain S.100 was effective in reversing inhibition by caldesmon of skeletal-muscle actin (⌓), yet had no effect on caldesmon inhibition of smooth-muscle actin (△). In the presence of 1 mM-EGTA, S.100 had no effect (▬, ▲). Assay tubes contained 0.4 mg of skeletal-muscle myosin/ml, the MgATPase activity of which was activated by 38 μM actin, either from skeletal muscle (100% = 161 nmol/min per mg) or aorta (100% = 181 nmol/min per mg) with 9.4 μM aorta tropomyosin. Caldesmon (6 μM) inhibited MgATPase rate of either actin by approx. 66%.

S.100 was much less dependent on temperature and ionic strength. Thus, at 25 °C in buffer containing 70 mM-KCl, 20 μM-S.100 reversed caldesmon's inhibition of skeletal-muscle actin, but not of smooth-muscle actin (Fig. 3). This result was verified with two independent preparations of actin, caldesmon and S.100.

Reconstitution of Ca^{2+}-sensitive thin filaments with smooth-muscle Ca^{2+}-binding proteins

The protein of M_r 17000 from sheep aorta conferred Ca^{2+}-sensitivity on a synthetic thin-filament system consisting of smooth-muscle actin, tropomyosin and caldesmon. The behaviour of this protein under different experimental conditions was the same as that of brain S.100 or calmodulin (Fig. 4).

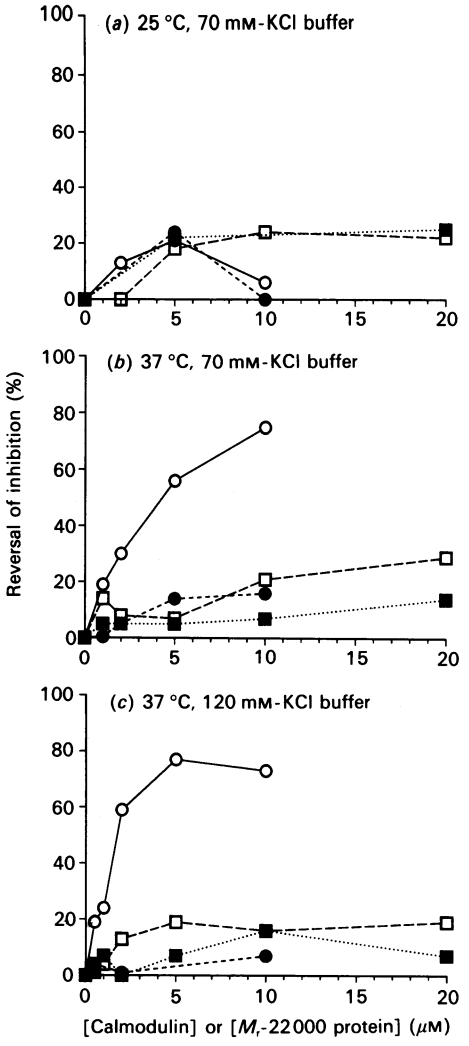

Fig. 4. Ca^{2+}-sensitizing activity of protein from sheep aorta

Two proteins from sheep aorta, one of M_r 17000 (○, ●) considered to be calmodulin, and one of M_r 11000 (□, ■) (similar to 'SMCaBP-11' of native M_r 22000), were tested for ability to reverse the inhibition by caldesmon of actin–tropomyosin-activated myosin MgATPase, in the presence of Ca^{2+} (○, □). The protein of M_r 17000 behaved like brain calmodulin under three sets of experimental conditions (see Table 1 for comparison). Symbols ● and ■ indicate presence of 1 mM-EGTA. Assay conditions are given in Fig. 2 legend.

The abundant protein of M_r 11000, from the same preparation, had no significant effect on ATPase rate under any conditions used (Fig. 4). Two independent preparations of this protein were made from sheep aorta; the behaviour of the proteins in the ATPase assay was similar in either case.

The third protein, of M_r 9000, was tested at up to 24 μM in buffer containing 120 mM-KCl at 37 °C, but had no effect on ATPase rate in either the presence or the absence of Ca^{2+} (results not shown).

DISCUSSION

Brain S.100 interaction with caldesmon

Brain S.100, like brain calmodulin, conferred Ca^{2+}-sensitivity on a reconstituted smooth-muscle thin-filament system. S.100 binds to caldesmon in the presence of Ca^{2+} [22,23]; presumably, the Ca^{2+}·S.100–caldesmon complex no longer inhibits actin–

tropomyosin. In our experiments, inhibition by 2–3 μM-caldesmon was, under optimal conditions, reversed by approximately stoichiometric amounts of S.100. Thus the binding constant between S.100 and caldesmon is probably at least 10^6 M^{-1}. We did not test S.100 isoforms separately, but our preparation contained mainly β-subunit, as reported for bovine brain [20,21,25], so its activity probably involved dimers containing that subunit. The possible activity of the α-subunit cannot be assessed from our data. The pattern of dependence on temperature and ionic strength is identical with S.100 or calmodulin [9]. Binding studies have clarified why calmodulin behaves in this way. At 37 °C, a Ca^{2+}·calmodulin–caldesmon–actin–tropomyosin complex can be formed, which activates myosin MgATPase fully [16]. At 25 °C this complex is not formed; reversal of caldesmon inhibition then depends on the competition for binding to caldesmon between Ca^{2+}·calmodulin (the binding constant between Ca^{2+}·calmodulin and caldesmon is approx. 10^6 M^{-1}) and actin–tropomyosin (the binding constant between caldesmon and actin–tropomyosin is approx. 10^7 M^{-1}), and consequently a large excess of calmodulin is required [9,16]. It seems likely that the mechanism of S.100-mediated Ca^{2+}-sensitivity is both qualitatively and quantitatively similar. It appears to be quite unusual for calmodulin and S.100 to interact with the same target protein in such a similar fashion [18–20].

We have considered that the calmodulin–caldesmon interaction does not account for the Ca^{2+}-sensitivity of native thin filaments [13,15]. Skripnikova & Gusev [22] reported that S.100 could regulate reconstituted thin fialments at 25 °C in buffer of low ionic strength, but calmodulin did not. They suggested that S.100 might be the intrinsic Ca^{2+}-sensitizing factor of native thin filaments. We also found that, if thin filaments were reconstituted with skeletal-muscle actin, smooth-muscle tropomyosin and caldesmon, then S.100 could indeed reverse caldesmon inhibition at 25 °C. However, this also occurred with brain calmodulin, and is a result of the greater tendency for caldesmon to bind to skeletal-muscle actin without inhibiting it [9,11,12]. Since caldesmon and skeletal-muscle actin are unlikely to interact in cells, this observation may not be physiologically significant. We cannot account for the lack of activity of calmodulin in the experiments of Skripnikova & Gusev [22], except that their source of caldesmon and other experimental details differ from ours. Our data do not indicate that reconstitution of thin filaments with S.100 rather than calmodulin produces a better model of the native filaments.

Smooth-muscle S.100-like proteins and caldesmon

The caldesmon content of smooth muscle is probably about 18 μM [32]. Thus any Ca^{2+}-binding protein which regulated caldesmon in smooth-muscle cells would have to be present at a comparable level; calmodulin concentrations may be up to 30 μM [33,34]. Immunochemical studies have detected only low amounts of S.100 in smooth muscles [20,21]. However, other S.100-like proteins might be present in smooth muscle, but might not cross-react with antibodies to brain S.100.

Hydrophobic-interaction chromatography is a well-known technique for preparing Ca^{2+}-binding proteins such as calmodulin or annexins for smooth muscle [35]. The fraction of smooth-muscle proteins eluted from phenyl-Sepharose with EDTA contained numerous protein species, presumably including potential Ca^{2+}-binding proteins such as calmodulin. Our attempts to prepare smooth-muscle S.100 produced three protein species, none of which was identical with brain S.100 isoforms.

One of these proteins was of the same M_r as calmodulin (17000), had the same chromatographic properties, and behaved in exactly the same way as brain calmodulin in the reconstituted thin-filament system. It seems highly likely that this protein was smooth-muscle calmodulin; our data do not support the theory [36,37] that the Ca^{2+}-binding protein of M_r 17000 in smooth muscle is different from calmodulin. The other two proteins were of low M_r, like S.100, but both were different from S.100 in their mobility on SDS/polyacrylamide gels, and in their chromatographic behaviour on anion-exchange. Both proteins partially cross-reacted with an antiserum to mouse parvalbumin (J.-M. Gillis, personal communication), suggesting some homology with parvalbumins, and possibly other EF-hand proteins. Parvalbumin as such may be absent from smooth muscle [38]. Oncomodulin has a similar M_r (reported as 11700), and is closely related to parvalbumins [39], though it has not been shown to occur in normal adult tissues [40]. There are several other Ca^{2+}-binding proteins of similar M_r, including the calgranulins [41], the M_r-10500 calcyclin-like protein [42] and calbindin '9k' (M_r 8800; [43]), but the reported distribution of these proteins does not include smooth muscle. The protein of M_r 11000 may by the SMCaBP-11 recently reported by Mani & Kay [44], since the apparent M_r, abundance and elution from anion-exchange were similar. Is has been suggested that this protein may have a regulatory role [44]. However, its functions probably do not include regulation of caldesmon, since it had no effect on ATPase in our reconstituted thin-filaments system, even though the protein of M_r 17000 was active, suggesting that the preparation method did not destroy activity. The identity of the protein of M_r 9000 is more uncertain, as it does not seem to correspond to any reported smooth-muscle protein; this protein did not appear to interact with caldesmon. We found no evidence, therefore, that aorta smooth muscle contained a sufficient concentration of S.100 to regulate caldesmon in the thin filaments. Nevertheless, S.100 is potentially an efficient regulator of the caldesmon–actin interaction under physiological conditions. Some types of non-muscle cells may contain both S.100 and the lower-M_r caldesmon isoform, and caldesmon–S.100 interactions could be important in regulating cellular functions in such cells. Neither of the low-M_r proteins which we isolated from aorta had any effects on caldesmon in reconstituted thin filaments, but these negative results do not, of course, rule out the existence of any alternative regulatory protein which might be involved in controlling smooth-muscle actomyosin.

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