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Anti-actin antibodies

An immunological approach to the myosin–actin and the tropomyosin–actin interfaces

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The topography of the rigor complex between subfragment-1 (S-1) of myosin and actin was investigated by using several specific antibodies directed to well-located sequences in actin. A major contact area for S-1 was characterized in the hydrophilic 18–28 constant sequence, and the variable 1–7 sequence was only found to be in close proximity to the interface. The *C*-terminal extremity of actin situated around Cys-374 appeared to be included in a region close to the S-1 heavy chain and the *N*-terminal part of actin. The interaction between tropomyosin and actin was also studied. Neither of the terminal parts of actin were involved in this interaction. Thus, the regions involved in the interactions of S-1 and tropomyosin with actin do not overlap.

INTRODUCTION

Muscle contraction results from thick filaments (myosin) and thin filaments (actin and essentially regulatory proteins such as troponins and tropomyosin) sliding past each other. Tropomyosin lies in the grooves on each side of the actin helix. The sliding process requires cyclic interactions between myosin heads and actin monomers on the microfilament (Huxley, 1969). Myosin affinity for actin depends on the kinetic steps of ATP hydrolysis by myosin heads. The most stable complex is formed in the absence of nucleotides (Lymm & Taylor, 1971). It is known as the 'rigor complex'. Tropomyosin–troponin complex modulates these interactions. The regulatory effect may result either from a steric blocking effect or from long-range effects on myosin ATPase activity (Adelstein & Eisenberg, 1980).

The supramolecular organization of the myosin subfragment-1 (S-1)–actin complex and the tropomyosin–actin complex is well known at low resolution from three-dimensional image reconstructions (Egelman, 1985). However, the precise topology of the interacting regions on the actin molecule has only been deduced from indirect investigations. ^{1}H-n.m.r., chemical modifications, crosslinking and protein cleavage have been widely used (see Hambly *et al.*, 1986, for a review). It has been shown that the S-1 heavy chain interacts with some regions on the *N*-terminal part of actin (Levine & Moir, 1985; Moir *et al.*, 1986; Sutoh, 1982), and the myosin light chain A_1 can be crosslinked to actin by ethyl(diaminopropyl)carbodi-imide on the *C*-terminal sequence of actin (the 360–363 sequence) (Sutoh, 1982) and by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (Labbé *et al.*, 1986). The interface between actin and tropomyosin is not well known (Hambly *et al.*, 1986) although the 70–86 and 340–375 sequences have been postulated as possible contact regions (Johnson & Stockmal, 1982).

In previous papers (Benyamin *et al.*, 1986; Roustan *et al.*, 1986), we have described the preparation of two different anti-actin antibody populations mapping the *N*-terminal 1–7 and 18–28 sequences respectively. The *C*-terminal extremity can also be studied by using anti-dansyl antibodies specific for the chemically labelled Cys-374 of actin (Boyer *et al.*, 1985). In the present paper, we describe the use of these specific antibodies to probe the binding sites of S-1 and tropomyosin in actin. We delimit a precise region on the *N*-terminal of actin related to the S-1 interaction; the *C*-terminal extremity is probably located near this interface. A preliminary report related to the S-1–actin interface has previously been published (Mejean *et al.*, 1986).

MATERIALS AND METHODS

Proteins and chemically modified derivatives

Rabbit skeletal muscle actin was purified (Spudich & Watt, 1971) from acetone powder. Myosin was isolated from rabbit skeletal muscle according to Offer *et al.* (1973). S-1 was prepared by digestion of myosin with chymotrypsin (Weeds & Taylor, 1975) and purified (Labbé *et al.*, 1981) by gel filtration over Sephacryl S-200. The isoenzymes S-1 A_1 and S-1 A_2 were isolated after DEAE cellulose chromatography (Weeds & Taylor, 1975). Tropomyosin from rabbit skeletal muscle was purified according to Cavadore *et al.* (1985).

Actin was *S*-carboxymethylated as previously described (Benyamin *et al.*, 1986). Actin was labelled at Cys-374 by 1,5-IAEDANS according to Takashi (1979). S-1 was iodinated with ^{125}I by using the Iodogen method (Fraker & Speck, 1978). Its specific radioactivity was 20000 c.p.m./μg.

Actin cleavage

Actin was proteolysed with trypsin (Jacobson & Rosenbusch, 1976) and thrombin (Muszbek & Laki,

Abbreviations used: S-1, myosin subfragment-1; F-actin, filamentous actin; A_1, alkali light chain 1; A_2, alkali light chain 2; Fab, immunoglobulin G fragment; Ab, antibodies; 1–7 antibodies, anti-[actin-(1–7)-sequence] antibodies; 18–28 antibodies, anti-[actin-(18–28)-sequence] antibodies; e.l.i.s.a., enzyme-linked immunosorbent assay; 1,5-IAEDANS, *N*-(iodoacetyl)-*N'*-(5-sulpho-1-naphthyl)ethylenediamine.

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1974). The *N*-terminal thrombic peptides (actin peptides 1–28 and 29–39) (Muszbek & Laki, 1974) were isolated by h.p.l.c. Thrombic digest was fractionated on a μBondapack C_{18} column (Waters) (7.8 mm × 300 mm) with a 0–60% (v/v) acetonitrile gradient in 0.01% (v/v) trifluoroacetic acid (Roustan *et al.*, 1986).

Antibodies

Antibodies to rabbit F-actin were obtained as described by Benyamin *et al.* (1986). Anti-IgG antibodies labelled with alkaline phosphatase were from Biosys (France). Antibody populations were purified by affinity chromatography on insolubilized *S*-carboxymethylated actin, F-actin or small *N*-terminal tryptic peptides, as previously described (Benyamin *et al.*, 1986). Sheep anti-dansyl serum was elicited as previously described (Boyer *et al.*, 1985). The purified antibody and the corresponding Fab (Benyamin *et al.*, 1983) were purified by affinity chromatography (Boyer *et al.*, 1985).

Analytical methods

Protein concentrations were determined by absorbance (Mejean *et al.*, 1986; Woods, 1967). SDS (0.1%)/polyacrylamide-slab-gel electrophoreses were run according to the procedure of Laemmli (1970). Cosedimentation experiments were carried out using a Beckman Airfuge. F-actin or dansylated F-actin (50 μM) were incubated for 10 min with tropomyosin (7 μM) in 2 mM-Tris/HCl buffer (pH 7.5) containing 0.1 mM-$CaCl_2$, 0.2 mM-ATP, 2 mM-$MgCl_2$ and 0.1 M-KCl. After ultracentrifugation (30 min at 100000 *g*), the pellets were analysed by slab-gel electrophoresis. The same amount of tropomyosin (determined by densitometry) was cosedimented with F-actin or dansylated F-actin (results not shown). Thus, dansylation of Cys-374 in F-actin did not prevent the interaction with tropomyosin.

The e.l.i.s.a. technique (Engvall, 1980) was used to monitor the interaction between actin and antibody populations in the presence of S-1 or tropomyosin.

In the direct method, 50 μg of filamentous actin in 150 μl of 0.5 M-Na_2CO_3/$NaHCO_3$ (pH 9.6)/0.01% NaN_3 was added to the wells of 96-well U-bottom microtitre plates. After overnight incubation at 4 °C, excess actin was removed and the plates were automatically washed (Titertek Microplate Washer 120; Flow Laboratories) with 0.15 M-NaCl/0.15 M-KCl/10 mM-Na_2HPO_4 (pH 7.4)/0.05% Tween. The plates were incubated with 0.5% (w/w) gelatin + 3% (w/w) gelatin hydrolysate in 10 mM Tris/HCl/100 mM-KCl buffer, pH 7.4, to saturate all the free protein binding sites. After washing with 10 mM-Tris/HCl/100 mM-KCl buffer, pH 7.4 (supplemented with 5 mM-$MgCl_2$ in the case of tropomyosin), the plates were incubated for 2 h at 37 °C with a selected dilution of antibodies or S-1 (or tropomyosin) in the presence of increasing concentrations of S-1 (or tropomyosin) or antibodies. After four washes with the Tween buffer, the plates were incubated for 1 h at 37 °C with anti-(rabbit IgG) [or anti-(sheep IgG)] labelled with alkaline phosphatase and diluted to 1:3000. After washing with 0.15 M-NaCl/10 mM-Na_2HPO_4 (pH 7.4)/0.05% Tween, a solution of *p*-nitrophenol phosphate (1 mg/ml) was added to the wells and the mixture was incubated for 30 min at room temperature. The resulting absorbance at 405 nm was read automatically with a Titertek Multiskan Plus Instrument (Flow Laboratories). Each sample was assayed in triplicate and the mean value was calculated. Non-specific absorption was determined for each sample under the same conditions using deactivated uncoated wells.

In the indirect method, *S*-carboxymethylated actin (50 μg) was coated under the same conditions as above. Free antigen (actin peptide 29–39, F-actin or tropomyosin–F-actin complex) was added just before the addition of the first antibody and the process continued as described above. Specific details are given in the Figure legends.

RESULTS

Involvement of the *N*-terminal part of actin in S-1 and tropomyosin interactions

Analysis of the antigenic structure of F-actin has shown the presence of two epitopes in the *N*-terminal sequence (1–39) (Roustan *et al.*, 1986). The first is located in the variable 1–7 sequence, and the second in the constant hydrophilic 18–28 sequence. In the present work the hydrophobic 29–39 sequence appeared to be excluded from the antigenic structure, since the related thrombic peptide (actin peptide 29–39) was unable to interact with anti-F-actin antibodies (results not shown).

Thus, two purified antibody populations directed to the 1–7 and 18–28 sequences were used to study the interaction of S-1 (rigor complex) on the *N*-terminal part of actin by direct e.l.i.s.a. In a previous report (Mejean *et al.*, 1986), we determined an apparent K_D of S-1 for coated F-actin (K_D 0.45 μM) on microtitre plates which essentially agrees with those reported in homogeneous phase. These antibodies were also used to study tropomyosin interactions by direct and indirect e.l.i.s.a. techniques.

The 1–7 sequence and S-1 binding

In the first e.l.i.s.a. experiment, various concentrations of antibodies to the 1–7 sequence (1–7 antibodies) were incubated in the presence of S-1 fixed at a saturating concentration ($20 \times K_D$). The amounts of antibodies interacting with coated F-actin were measured at 405 nm. As shown in Fig. 1(*a*), the binding of specific antibodies was partially affected by the presence of S-1 on F-actin. When we varied the S-1 concentrations at a fixed antibody concentration and measured antibody binding to coated F-actin, a biphasic response was observed (Fig. 1*b*). At low S-1 concentrations ($< 0.3 \times K_D$) we observed an increase in antibody binding, which returned to slightly below the initial binding when the S-1/actin ratio increased. We also studied the binding of antibodies at various S-1 and antibody concentrations. The results are shown in Fig. 1(*c*). A plot of $1/A_{405}$ versus 1/[antibodies] shows that the maximum amount of antibodies able to react with F-actin was not S-1-dependent. The variation in the apparent K_{Ab} (Fig. 1*d*) is in accordance with the curve profile in Fig. 1(*b*), showing an increase in affinity at low S-1 concentrations. Furthermore, this apparent affinity only differed by a factor of two in the absence of S-1 or at saturating S-1 concentrations.

Moreover, when S-1 was ^{125}I-labelled and its interaction with actin measured in the presence of increasing antibody concentrations, the binding of S-1 appeared to be only slightly affected by the 1–7 antibody (Fig. 2).

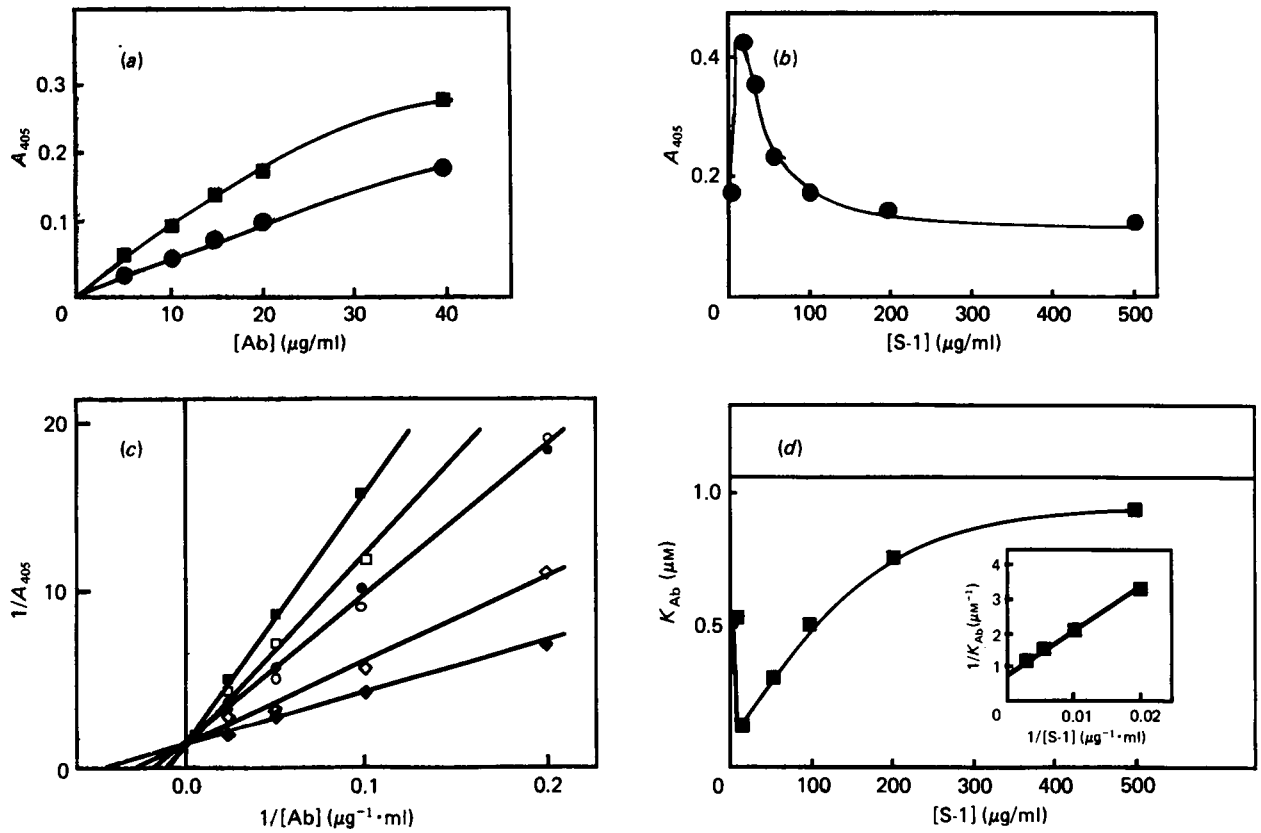

Fig. 1. Reactivity of antibodies specific for the *N*-terminal sequence of actin (1–7 sequence) in the presence of S-1

Direct e.l.i.s.a. were carried out as described in the Materials and methods section. The binding of specific anti-actin antibodies was monitored at 405 nm using anti-IgG antibody labelled with alkaline phosphatase. (*a*) Specific antibodies (Ab) were reacted with coated F-actin in the presence (●) or absence (■) of S-1 at a saturating concentration (1 mg/ml). (*b*) Specific antibodies (20 μg/ml) were reacted with coated F-actin in the presence of increasing S-1 concentrations (between 0 and 500 μg/ml). (*c*) Specific antibodies at various concentrations (between 5 and 40 μg/ml) were reacted with coated F-actin in the presence of several S-1 concentrations. A plot of $1/A_{405}$ versus 1/[Ab] was drawn at the following S-1 concentrations: ○, 0 μg/ml; ◆, 10 μg/ml; ◇, 50 μg/ml; ●, 100 μg/ml; □, 200 μg/ml; ■, 500 μg/ml. (*d*) Apparent dissociation constants for antibodies (K_{Ab}) estimated from results in (*c*) are plotted versus S-1 concentrations. Inset: replot of $1/K_{Ab}$ versus 1/[S-1].

The 18–28 sequence and S-1 binding

A similar approach was used to investigate the involvement of the 18–28 sequence in the *N*-terminal part of actin.

In contrast with the above results, this second specific antibody population (18–28 sequence) was almost completely excluded by high S-1 concentrations (Fig. 3*a*). The antibody binding also depended on the amounts of S-1 in the reaction mixture (Fig. 3*b*) and was equally enhanced at low S-1 concentrations (Figs. 3*b* and 3*c*). The corresponding apparent dissociation constants (K_{Ab}) were very high at saturating S-1 concentrations and their variations at low S-1 concentrations (Fig. 3*d*) were in agreement with the profile in Fig. 3(*b*).

Finally, when ^{125}I-labelled S-1 was used, increasing concentrations of the antibody to the 18–28 sequence (18–28 antibody) provoked the release of ^{125}I-S-1 from coated F-actin (Fig. 2).

The 1–28 sequence and tropomyosin binding

In previous work (Benyamin *et al.*, 1986; Cavadore *et al.*, 1985), we reported that the antiserum induced by F-actin used in this work has two kinds of antibody populations. The first is directed to sequential epitopes (1–7 and 18–28 sequences), and the second is specific for the F-actin conformation (Cavadore *et al.*, 1985), and could not be related to a region in the actin sequence.

By using the direct e.l.i.s.a. technique, the binding of these antibody populations to coated F-actin was monitored in the presence of various tropomyosin concentrations. As shown in Fig. 4, a significant inhibitory effect (about 60%) was observed in the case of antibodies specific for the F-actin conformation. Thus, the reactivity of these antibodies seemed to be affected by the presence of tropomyosin on F-actin or by a related conformational change. In contrast, the reactivity of the antibody population directed to the sequential epitopes (1–7 and 18–28) was not altered in the presence of tropomyosin (Fig. 4). This last result, confirmed (results not shown) by indirect e.l.i.s.a. (see the Materials and methods section) using the tropomyosin–actin complex (1:7 mol/mol) as the free antigen, excludes the 1–28 sequence from the tropomyosin–F-actin interface.

Involvement of the *C*-terminal extremity of actin in S-1 or tropomyosin interactions

As the *C*-terminal extremity of actin is located near its *N*-terminus (Boyer *et al.*, 1985; Sutoh & Mabuchi, 1984),

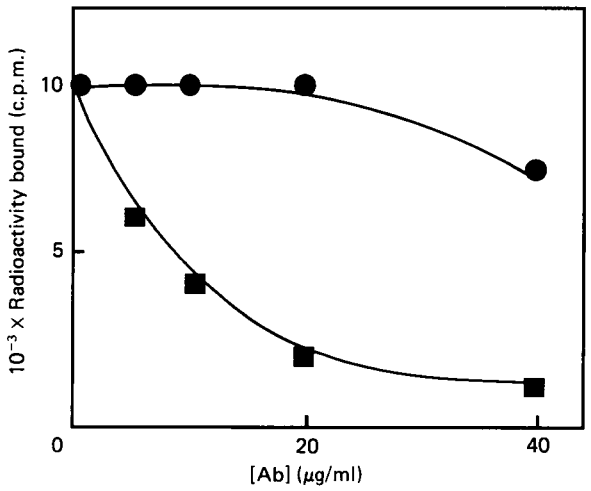

Fig. 2. Effect of specific anti-actin antibodies towards S-1 binding to coated F-actin

^{125}I-S-1 was reacted with coated F-actin (50 μg) in microtitre plates in the presence of various antibody concentrations (as indicated) in 10 mM-Tris/HCl/100 mM-KCl buffer pH 7.4. After washing with the same buffer, the amount of ^{125}I-S-1 in interaction with F-actin in the wells was counted in a Packard model 5105 Autogamma spectrometer. The results are expressed as the radioactivity bound versus 1–7 antibody (●) or 18–28 antibody (■) concentrations.

we studied a possible relationship between the region around Cys-374 and S-1 binding to the 18–28 sequence. Actin can be modified at Cys-374 by 1,5-IAEDANS without preventing interactions with S-1 (Takashi, 1979) or tropomyosin (see the Materials and methods section). Moreover, the actin modified at Cys-374 is able to interact with anti-dansyl antibodies (Boyer *et al.*, 1985). As shown in Fig. 5(*a*), the interaction of anti-dansyl antibodies with dansylated actin was prevented by the presence of S-1. The response was different from that obtained with the *N*-terminal population and showed only a decrease in antibody interaction versus S-1 concentrations (Fig. 5*a*). In addition, almost all the antibodies were released (> 90%) at high S-1 concentrations (> 5 × K_D). Similar results were obtained with Fab, which are not as bulky (Fig. 5*a*). The binding of Fab at various S-1 and Fab concentrations was also studied (results not shown). A plot of $1/K_{Fab}$ versus 1/[S-1] shows (Fig. 5*a*, inset) that the apparent dissociation constants strongly increase in the presence of S-1. It is also known from crosslinking experiments (Sutoh, 1982; Labbé *et al.*, 1986) that light chain A_1 may interact with some parts of the *C*-terminus (particularly the 361–364 sequence).

We tested for possible competition between anti-dansyl antibodies and two kinds of S-1 possessing light chains A_1 or A_2 for dansylated F-actin. As shown in Fig. 5(*b*), the two entities provoked an equal release of antibodies from F-actin.

The binding of the anti-dansyl antibody population to coated dansylated F-actin was also measured in the presence of increasing amounts of tropomyosin. Once more, tropomyosin did not modify the antibody interaction (Fig. 4). Thus, the *C*-terminal extremity of actin would appear to be excluded from the actin–tropomyosin contact region.

DISCUSSION

The interaction of F-actin with S-1 is correlated with a large enhancement of myosin ATPase activity. During this interaction, actin monomers in the microfilament bind strongly to the myosin head in a stable state called 'rigor'. A structural communication between ATP in the catalytic site of S-1 and actin has recently been located in a 30 kDa *C*-terminal fragment of S-1 (Chaussepied *et al.*, 1986). This fragment seems to contain the two supposed actin sites for the heavy chain, which have previously been narrowed down to the central 50 kDa and *C*-terminal 20 kDa fragments of S-1 (Hiratsuka, 1986; Mornet *et al.*, 1981). The light chain A_1 is also involved in these interactions (Levine & Moir, 1985).

Thus, identification of the corresponding binding region of S-1 in the actin primary structure is an important goal. The spatial organization of actin–S-1 is known only at low resolution which does not yield a precise description of the interface. Part of the interface has been situated within the 1–44 sequence of actin (Moir *et al.*, 1986). The reactivity of Lys-373 is also modified in the complex. This perturbation may be related either to a conformational change induced by S-1 or to a steric effect, since this lysine residue is not affected by binding contacts (Szilagyi & Lu, 1982). Furthermore, as the two extremities of the actin monomer are somewhat close to one another (Boyer *et al.*, 1985; Sutoh & Mabuchi, 1984), they could be included in the same interface between actin and S-1.

Our results, which are in agreement with those obtained by others using ^{1}H-n.m.r. or crosslinking reagents, indicate that the *N*-terminal sequence of actin lies near or at the S-1 interaction site (Sutoh, 1982; Moir *et al.*, 1986). Furthermore, our data provide some precise new structural and topological information. Thus, we observed that at low S-1 concentrations, the binding of S-1 to F-actin enhanced the antigenic reactivity of the 1–7 and 18–28 sequences (Figs. 1*b* and 3*b*). The decrease in the apparent dissociation constants for the 1–7 and 18–28 antibody populations (Figs. 1*d* and 3*d*) could be related to a greater exposure of this part of actin after a limited interaction of S1 on the microfilament. These large, long-range conformational changes along the microfilament have been reported by others (Ikkai *et al.*, 1979; Yanagida *et al.*, 1984). In this connection, Rouayrenc *et al.* (1985) have also reported that the binding of S-1 to F-actin influence at least four neighbouring actin subunits, and strengthens the interaction between the monomers. Moreover, the 1–7 sequence does not seem to be directly involved in the S-1 interaction, and the 18–28 sequence appears to be included in the binding site. This conclusion is based on an analysis of the simultaneous binding of S-1 and the 18–28 antibody population to F-actin, which displays a competition pattern. Thus, a ternary S-1–antibody–actin complex does not occur in the case of the 18–28 antibody population, but is obtained with the 1–7 antibody population. This contact region is in accordance with ^{1}H-n.m.r. information (Moir *et al.*, 1986), which shows that some residues in the region, particularly Phe-21 and -31 are perturbed during the S-1 interaction and are located close (< 1.2 nm) to the $-SH_1$ group of S-1. A steric hindrance between S-1

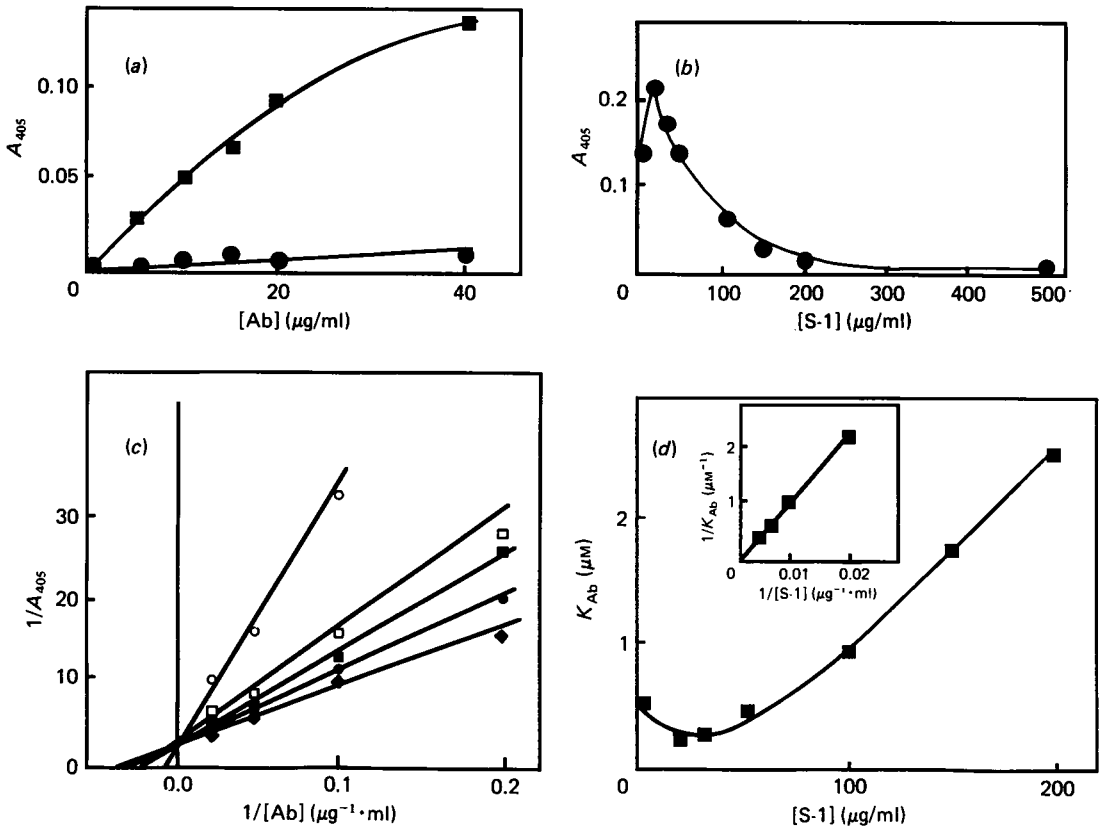

Fig. 3. Reactivity of antibodies specific for the *N*-terminal sequence of actin (18–28 sequence) in the presence of S-1

Direct e.l.i.s.a. were carried out as described in the Materials and methods section. (*a*) Specific antibodies were reacted with coated F-actin in the presence (●) or absence (■) of S-1 at a saturating concentration (1 mg/ml). (*b*) Specific antibodies (20 μg/ml) were reacted with coated F-actin in the presence of increasing S-1 concentrations (between 0 and 500 μg/ml). (*c*) Specific antibodies at various concentrations (between 5 and 40 μg/ml) were reacted with coated F-actin in presence of S-1 at the indicated concentrations. A plot of $1/A_{405}$ versus 1/[Ab] was drawn at the following concentrations: □, 0 μg/ml; ◆, 20 μg/ml; ●, 30 μg/ml; ■, 50 μg/ml; ○, 100 μg/ml. (*d*) Apparent dissociation constants for antibodies (K_{Ab}) are plotted versus S-1 concentrations. Inset: replot of $1/K_{Ab}$ versus 1/[S-1].

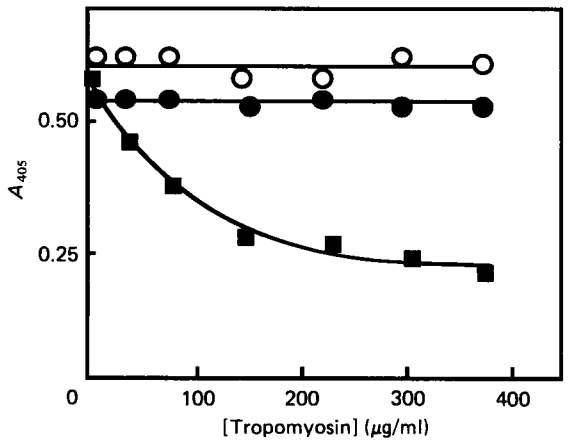

Fig. 4. Effect of tropomyosin on the reactivity of specific anti-actin antibody populations studied by direct e.l.i.s.a.

Experimental conditions were as described in the Materials and methods section. Tropomyosin was varied from 0 to 375 μg/ml. Reactivity of antibodies specific for conformational epitopes (20 μg/ml; ■), or for anti-*N*-terminal sequence (10 μg/ml; ○) towards coated F-actin; antibodies specific for the dansyl hapten (32 ng/ml; ●) towards coated dansylated F-actin.

and the 18–28 antibody populations on actin could explain the observed competition in the case where the antigenic epitope would be in spatial proximity to the actin–S-1 interface. The 1–7 sequence appears to lie near the S-1 interface, extending out of the actin molecule (Benyamin *et al.*, 1986) and allowing the well-known crosslinking of S-1 by ethyl(diaminopropyl)carbodi-imide (Mornet *et al.*, 1981) at residues 1–4 and 11. Other arguments can be given to show that this part of the *N*-terminal sequence is excluded from the S-1 binding interface. For instance, S-1 crosslinked by ethyl(diaminopropyl)carbodi-imide to the thrombic *N*-terminal 1–28 peptide by its acidic *N*-terminal amino acid residues [obtained by thrombic cleavage of the actin–S-1 ethyl(diaminopropyl)carbodi-imide complex], always interacts with F-actin without changes in its apparent dissociation constant and can be dissociated by pyrophosphate (Labbé, 1984). Moreover, vascular and stomach actins, which differ at their *N*-terminal extremities, show the same interaction parameters with S-1, but a relation between the mutation at residue 17 and activation of S-1 has been proposed (Mossakowska & Strzelecka-Golaszewska, 1985) to explain differences in activation properties between skeletal and smooth muscle actins.

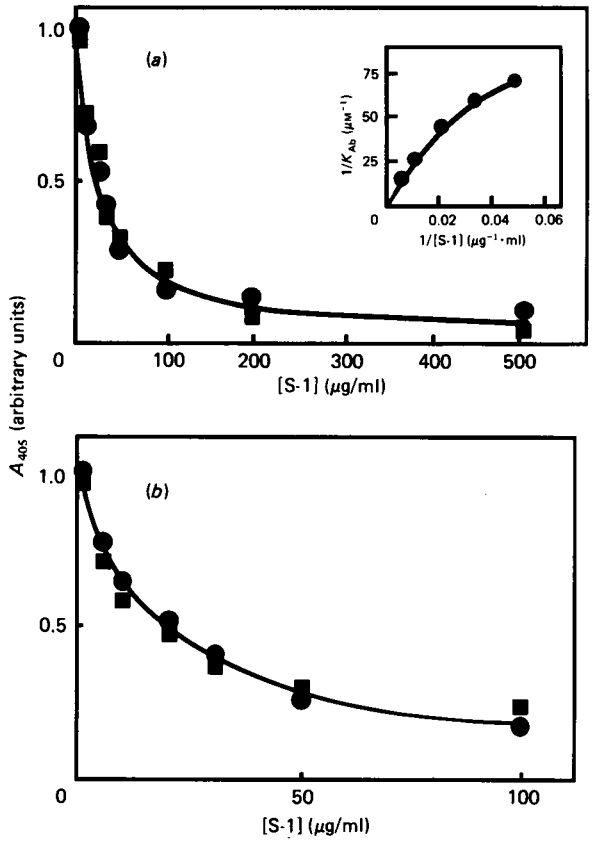

Fig. 5. Reactivity of anti-dansyl antibodies (or Fab) in the presence of S-1

S-1 concentrations were varied from 0 to 500 μg/ml. (*a*) Comparison of the results obtained with antibodies (■; 0.1 μg/ml) or Fab (●; 0.4 μg/ml). Inset: $1/K_{Fab}$ is plotted versus 1/[S-1]. (*b*) Comparison of the reactivity of anti-dansyl antibodies (0.1 μg/ml) in the presence of the two purified isoforms of S-1: S-1 A_1 (■) or S-1 A_2 (●).

The relation between the *C*-terminal extremity and the S-1 binding site was also studied. We observed a release of antibodies or Fab directed to dansylated Cys-374 in the presence of S-1 (Fig. 5*a*). This result could be explained by the proximity of this residue to the S-1 bound to actin (Mossakowska & Strzelecka-Golaszewska, 1985). Thus, the presence of S-1 on actin increases the apparent dissociation constant of anti-danysl Fab (Fig. 5*a*, inset). However, a simple model assuming only a direct competition between S-1 and Fab cannot fit our results. An alternative interpretation is local alterations of the environment around Cys-374 induced by S-1 binding. These structural changes, probed by a number of studies using fluorescent labels (Criddle *et al.*, 1985; Mossakowska & Strzelecka-Golaszewska, 1985), could affect the accessibility of the dansylated Cys-374 to antibodies or derivatized Fab. The possibility that this effect is related to the light chain A_1 of S-1, which is known (Sutoh, 1982) to interact around residue 362, can be excluded. Substitution by light chain A_2, which lacks the interacting part, did not modify the Fab release (Fig. 5*b*). It is more likely that the spatial proximity of Cys-374 to the *N*-terminal sequence of actin (Boyer *et al.*, 1985; Sutoh & Mabuchi, 1984), where a major binding site of S-1 to actin is located, induces this effect. Attempts to locate another binding site of S-1 in the *C*-terminal part of actin (Levine & Moir, 1985; Sutoh, 1982; Szilagyi & Lu, 1982) are now necessary.

Finally, it has been shown that antibodies directed to the *N*-terminal sequence and the *C*-terminal extremity of actin, which compete with S-1 binding, are unable to affect the tropomyosin–actin interaction (Fig. 4). This result underlines the fact that the regions concerned with S-1 and tropomyosin interactions do not overlap and excludes these sequences from the tropomyosin interface. Further studies are needed to locate the groove in the microfilament in which tropomyosin lies.

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