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CATION BINDING SITES ON ACTIN : A STRUCTURAL RELATIONSHIP BETWEEN ANTIGENIC EPITOPES AND CATION EXCHANGE

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Summary : Divalent cations such as Mg^{2+} and Ca^{2+}, which bind specifically to actin, induce conformational changes that affect its antigenic structure. The distribution of antigenic epitopes on the sequence shows that these structural modifications involve epitopes related to monomer-monomer interfaces. In the N-terminal part, the 1-7 acidic extremity is not affected, in contrast with sequence 18-28. The ability of polycations such as diamine to modify the actin structure at concentrations below 0,1 µM strengthens the hypothesis that in vivo these compounds act locally and specifically on actin polymerization.

Actin is known to be essential for motility and cytoskeletal organization. Its polymorphism as well as its ability to interact with a number of proteins is well documented (1). Early in vitro investigations (2) gave evidence for the effects of salts, particularly divalent cations (used in the millimolar range) on actin conformation (3) and the nucleation-elongation process (4, 5). Actin possesses a high affinity divalent cation binding site ($K_A = 10^8$-10^9 M^{-1}) and a number (5-9) of unlocalized lower affinity binding sites ($K_A = 10^3$-10^4 M^{-1}) which promote polymerization (5). It has frequently been argued that Ca^{2+}, in contrast with Mg^{2+}, induces a weakening of the polymeric structure of actin at mM concentrations (6). An increased sensitivity to shearing forces was observed

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Abbreviations used : (1-7) antibodies, anti-actin-(1-7)-sequence antibodies ; (18-28) antibodies, anti-actin-(18-28)-sequence antibodies ; (40-113) antibodies, anti-actin-(40-113)-sequence antibodies ; (168-226) antibodies, anti-actin -(168-226)-sequence antibodies ; (285-375) antibodies, anti-actin-(285-375) -sequence antibodies ; e.l.i.s.a., enzyme-linked immunosorbent assay. K (actin fragment : 40-375 sequence), L (actin fragment : 114-375 sequence), M (actin fragment : 40-113 sequence).

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(7) as well as a marked depolymerization in the case of yeast actin (8). A greater susceptibility of Ca^{2+}-treated filamentous actin to proteolysis has been proposed (9). In contrast, other cations such as polyamines (10, 11) have been described as efficient polymerization effectors able to bundle microfilaments. A physiological role for these polycations has been proposed in relation to microfilamentous structures in cytokinesis (10) and spermatogenesis (11).

In a previous study (12) we gave evidence for a conformational change related to Ca^{2+} - Mg^{2+} substitution in actin, affecting the exposure of antigenic epitopes on the 168-226 sequence. In the present, more exhaustive study, we used purified anti-actin antibody populations with different specificities for actin sequences as surface probes to detect and locate Ca^{2+}-, Mg^{2+}-, and polycations - induced conformational changes.

MATERIALS AND METHODS

Rabbit skeletal muscle and scallop adductor muscle actins were prepared as described in (13). Aortic smooth muscle actin (14) was a gift of Dr. J.C. Cavadore. (1-7) antibodies (15), (18-28) antibodies (16) and (285-375) antibodies (17) were purified under the conditions previously described. (168-226) antibodies were obtained from anti-actin sera induced in sheep by performic-acid oxidized actin (18). Such sera display selective activity against sequence (168-375) of actin (12, 13). The (168-226) antibodies were purified by affinity chromatography on Sepharose-4B-insolubilized 26kDa peptide (1-226 sequence) derived from S. aureus V_8 protease cleavage of actin (12). (40-113) antibodies were induced in rabbits by injection of the M peptide (40-113 sequence) (18) purified from a thrombic digest of actin and coupled to hemocyanine by glutaraldehyde (17). Antibodies were separated from antisera by affinity chromatography on Sepharose 4B-insolubilized M peptide immunosorbent (18). Their specificities were checked (Fig. 1A) by immunoblotting (18).

Direct e.l.i.s.a. (19) was previously described in detail (16). Each well was coated with 50 µg of filamentous actin polymerized in the presence of 2 mM Mg Cl_2, 0.1 mM ATP or 50 ng of monomeric actin purified in the presence of 0.1 mM $CaCl_2$, 0.1 mM ATP. The coated plates were washed and saturated (16) in the absence of divalent cations, after which they were incubated for 30 min at 37°C in 20 mM Tris buffer, pH 7.2, supplemented with 0.05 to 5 mM $CaCl_2$ or 0.05 to 5 mM Mg Cl_2 and 0.1 mM EGTA, or 0.002 to 10 µM spermine. After addition of the chosen dilution of the antibodies to the incubation mixture, the assay was carried out as described in (16). Each cation concentration was assayed in triplicate and the mean value of the observed effect was calculated.

RESULTS

Location of anti-actin antibody binding sites :

Two of the purified anti-actin antibody populations used in this work were previously found to be specific for sites on the 1-7 (15) and the 18-28 (16) sequences. The specificity of antibodies induced by the thrombic peptide (40-113 sequence) was tested by immunoblotting. As can be seen in Fig. 1B, these antibodies are not at all reactive with scallop actin, which differs from rabbit skeletal muscle actin by two mutations at positions 76

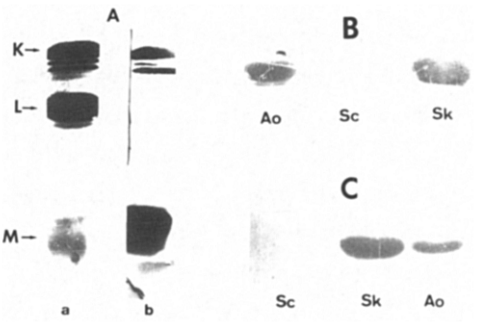

Fig.1 : Immunoreactivity of purified anti-actin antibody populations to various actins.
A. Reactivity of (40-113) antibodies with thrombic hydrolysate of actin.
(a) Coomassie-Blue-stained polyacrylamide gel after electrophoresis.
(b) Electrophoretic replicate revealed with antibodies.
B. Electrophoretic replicate of skeletal muscle (Sk), aortic muscle (Ao) and scallop muscle (Sc) actins revealed with (40-113) antibodies.
C. Electrophoretic replicate of skeletal muscle (Sk) aortic muscle (Ao) and scallop muscle (Sc) actins revealed with (168-226) antibodies.

and 103 (20). In contrast, aortic actin, which has one mutation at position 89 (20) afforded intact antigenic reactivity with these antibodies. This analysis was confirmed (results not shown) by quantitative e.l.i.s.a. Thus, the (40-113) antibody interaction sites appear to be related to position 76 and/or position 103 in the actin sequence. The specificity of the (168-226) antibodies can be narrowed down to antigenic epitopes located near residue 201. For instance, when Val was substituted for Thr in scallop actin (20) at position 201, the (168-226) antibodies lost all activity (Fig. 1C). A second variable residue, (Met 176), for which Leu is substituted in scallop actin (20), can be excluded from any antigenic region, since its oxidation in methionine sulfone is unable to affect (168-226) antibody reactivity (21). Finally, the specificity of the (285-375) antibodies was recently determined (22) and is located at positions including Met 299, Met 325 and Met 355.

Conformational changes induced by Mg^{2+} and Ca^{2+} :

The antibody populations described above were used in direct e.l.i.s.a. (see Materials and Methods) to monitor the antigenic reactivity of coated G- and F-actins in the presence of Ca^{2+} or Mg^{2+}. As shown in Table I, the N-terminal extremity of actin (1-7 sequence) showed unmodified activity in the presence of Mg^{2+} or Ca^{2+} at various concentrations (0.05 to 1 mM) with either the coated G- or F-conformomer of actin. Moreover, its antigenic activity was similar to that of EDTA-treated actin (results not shown). On the other hand, under the same conditions, the antigenic reactivity of the 18-28 sequence showed a decrease of $25 \pm 5\%$ when Ca^{2+} was substituted for Mg^{2+} on coated filamentous actin (Table I). The alteration produced by

TABLE I

Cation effects on anti-actin antibody interaction tested by the e.l.i.s.a. procedure

Coated-actins	G-actin			F-actin		
Divalent cations	Ca^{2+}	Mg^{2+}	Spermine	Ca^{2+}	Mg^{2+}	Spermine
1-7 sequence	0 %	0 %	0 %	0 %	0 %	0 %
18-28 sequence	0 %	0 %	0 %	25 $\pm$ 5 %	0 %	0 %
40-113 sequence	0 %	0 %	30 $\pm$ 5 %	0 %	25 $\pm$ 5 %	30 $\pm$ 5 %
168-226 sequence	0 %	25 $\pm$ 5 %	0 %	0 %	0 %	0 %
285-375 sequence	0 %	0 %	20 $\pm$ 5 %	0 %	25 $\pm$ 5 %	30 $\pm$ 5 %

The maximum loss (%) of antigenic reactivity is the mean of results from three independent experiments. The standard deviation is indicated.

exchanging Ca^{2+} with Mg^{2+} (in the presence of 0.1 mM EGTA) (Table I) in G-actin monitored by antibody interaction in the 201 sequence region confirms a previous detailed study (12).

In the case of the 40-113 and 285-375 sequences (Table I, Fig. 2A), the antigenic reactivity of related epitopes appeared to be markedly decreased (25 $\pm$ 5%) by the presence of Mg^{2+} on filamentous actin. Since the antibodies specific for these two regions are induced by unfolded 40-113 and 285-375 polypeptides, it is probable that this result indicates a local restructuring of F-actin in the presence of Mg^{2+}, but not in the presence of Ca^{2+} used at a similar concentration (Table I, Fig 2B). These local conformational changes modified the accessibility of the related antigenic regions. The antibody apparent dissociation constants were modified by a factor of about 1.5 in the presence of Mg^{2+} only in the case of (40-113) antibodies (Fig. 2B). In contrast, in the case of the (285-375) antibodies a decrease (about 25 %) in the amount of bound antibodies was observed (Fig. 2C).

Location of diamine binding sites on actin :

Spermine was selected as the polycation (diamine) in our study. Its efficacy as an actin polymerization effector, has been related by others

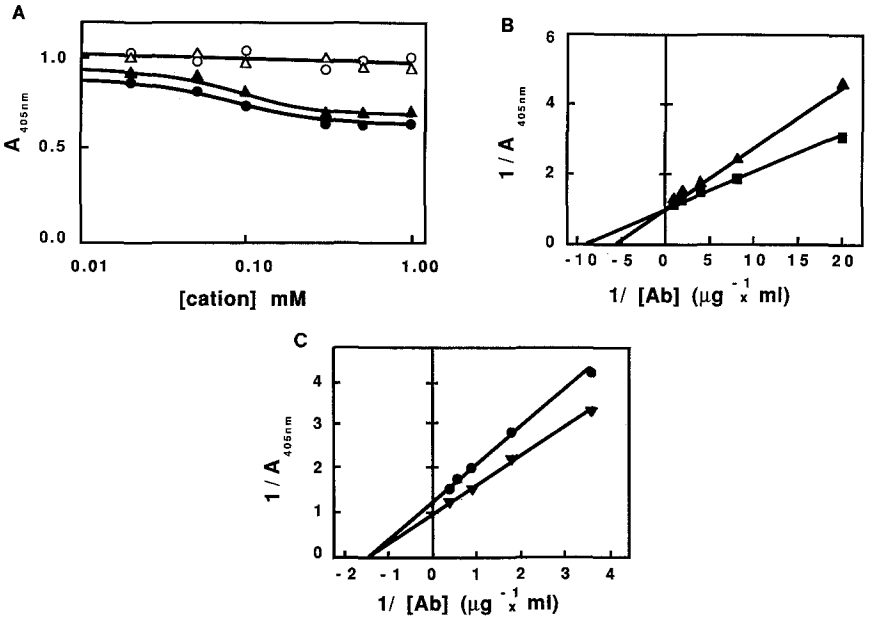

Fig.2 : Immunoreactivity loss revealed by e.l.i.s.a.
A. The (40-113) (▲,△) and (285-375) (●,○) antibodies were tested against filamentous actin in presence of Mg^{2+} (full symbols) or Ca^{2+} (open symbols).
B. Analysis of (40-113) antibody interaction with F-actin in the absence (■) or presence of 0.3 mM Mg^{2+} (▲).
C. Analysis of (285-375) antibody interaction with F-actin in the absence (▼) or presence of 0.5 mM Mg^{2+} (●).

(10) to its optimal chain length. As seen in Fig. 3, in the presence of spermine, antibodies specific for sequences 40-113 and 285-375 showed a marked decrease in their interaction with G- and F-actin. The effect was detected at 0,025 μM spermine and was almost complete at around 0,1 μM spermine. In contrast with Mg^{2+} and Ca^{2+}, coated G- and F-actins showed (Table I, Fig. 3) the same decreased reactivity in the presence of spermine and this effect was obtained at much lower effector concentrations (0.05-0.1 μM). Analysis of the effects (Fig. 3B and C) showed variation in antibody interaction parameters similar to that described above for Mg^{2+} on F-actin. In the case of spermine, the results were identical with G-actin (Fig.3) or F-actin (data not shown).

DISCUSSION Previous studies have shown that insolubilization (17, 23) and coating (16) of monomeric or filamentous actin have no significant effect on their binding properties to DNAse-I (17), gelsolin (22), tropomyosin (16) or myosin-subfragment-1 (16, 24). Moreover, conformational changes induced by myosin-subfragment-1 binding to filamentous actin (25) are easily detected by direct e.l.i.s.a. after actin coating (16). Consequently this approach, using selected anti-actin antibodies as

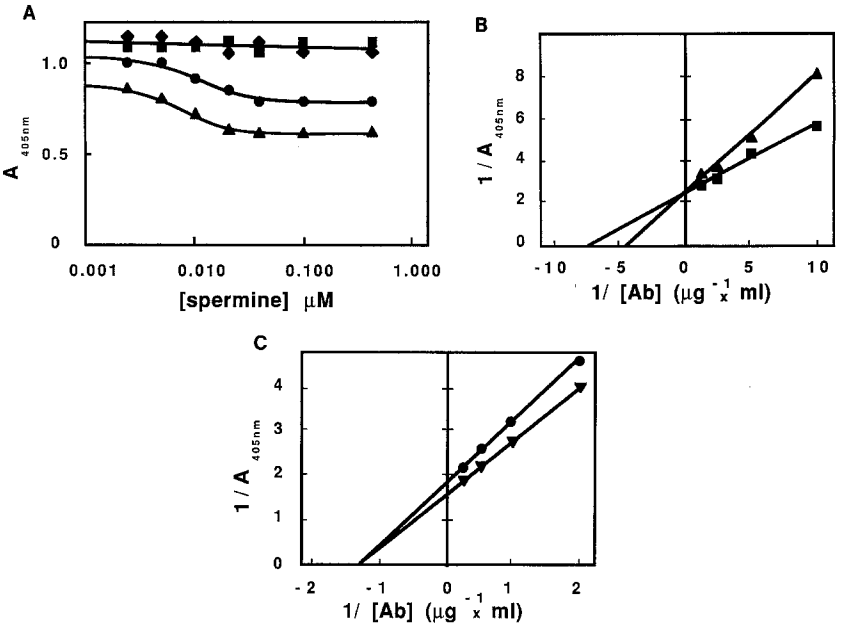

Fig.3 Immunoreactivity loss revealed by e.l.i.s.a.
A. (18-28) (■), (40-113) (▲), (168-226) (◆) and (285-375) (●) antibodies were tested against monomeric actin in the presence of spermine.
B. Analysis of (40-113) antibody interaction with G-actin in the absence (■) or presence of 0.1 µM spermine (▲).
C. Analysis of (285-375) antibody interaction with G-actin in the absence (▼) or presence of 0.1 µM spermine (●).

conformational indicators, was chosen to obtain information on cation binding to actin.

Our results show that the N-terminal extremity of actin (1-7 sequence) is not involved in a cation binding site, as has often been speculated (26, 27). Ca^{2+}- Mg^{2+} exchange, polymerization (28), unfolding of actin by S-carboxymethylation (28) or EDTA treatment (results not shown) were unable to affect the accessibility and reactivity of this sequence for (1-7) antibodies. These data are in good agreement with a number of studies (reviewed in (29)) based on the strong reactivity of this sequence with bulky reagents and its cross-linkage to different actin binding proteins. Hence, it is improbable that this variable sequence (20) is involved in the high affinity binding site for divalent cations ($K_A = 10^8$-10^9 M^{-1}), which is known to be a conserved structure (1) in actin evolution.

In contrast, other parts of actin, along sequences 18-28, 40-113, 285-375 and a region around Val 201, showed decreased antigenic reactivity when Ca^{2+} and Mg^{2+} were exchanged, at millimolar concentrations. This demonstrates that the binding of divalent cations at high and low affinity sites induces discrete restructuring which modifies exposed parts of the actin structure. It thus appears that the epitopes near the 76 and/or 103 residues become less accessible to antibodies and that only part of the

antigenic regions in the 285-375 sequence (22) become burried in the actin molecule upon Mg^{2+} and spermine binding.

The related epitopes involve regions implicated in the monomer-monomer interaction and actin proteolysis at sequence 40-113 and position 201 (17, 29) or actin severing (22, 30). Moreover the 18-28 sequence previously implicated in myosin binding (16) appears to be particularly sensitive to Ca^{2+} - Mg^{2+} substitution in the filamentous conformation of actin . Thus, the presence of Ca^{2+} at mM concentrations in the blood stream when actin is released (28) after tissue injury or cell death may favor actin severing by plasma gelsolin (31) and its clearance from circulation through some instability of the polymeric structure in a Ca^{2+} environment. Furthermore, the hypothesis (32) that diamine acts locally and specifically on the polymerization process in vivo, appears to be strengthened by our results showing a marked change of actin structure in the presence of 0.05 µM spermine.

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