Induction by chemically modified actin derivatives of antibody specificity

A relation between modified sites and antibody interactions with monomeric and filamentous actins

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A comparison of specific antibodies induced by unfolded actins modified either by oxidation or by arylation of lysine residues was reported. We have focused our work on binding properties with filamentous actin and located its preferential antigenic sites for the anti-arylated-actin antibodies in the C-part of the molecule. An interference of anti-oxidized actin antibodies upon actin polymerisation has also been reported.

Actin–Antibody interaction *Chemical modification* *Polymerisation* *Antigenic site*

1. INTRODUCTION

A recent increase of interest to antisera specific for actin [1–4] beside the question of microfilaments distribution is linked to the needful appreciation of structural and genetic polymorphisms of this protein through its cellular regulation.

A number of antisera obtained to detect actin has revealed various behaviours against the different actin states [1,2,5]. The various ways used to increase its poor immunogenicity, such as unfolding [6], chemical modification [7], aggregation [8] or use of human antibodies [5] could explain the absence of precipitating antibodies [9], a selective reactivity towards the filamentous form [1], or cross-reactivity with the unfolded and native monomeric or polymeric states [2,7].

To explore the relationship between physicochemical modifications of the actin molecule and the specificity of the antibodies induced, two different chemical modifications of actin have been carried out before unfolding by SDS treatment and immunization:

A physicochemical treatment by performic acid, oxidizing irreversibly the cysteine, methionine and tryptophan residues of actin which are located along the polypeptide chain [7];

A specific lysine arylation of F-actin by TNBS in mild conditions which maintains its polymeric form [10] and modified exposed residues [11].

Here we describe the binding properties of the induced antibodies with F-actin, the location of

Abbreviations: PAGE, polyacrylamide gel electrophoresis; buffer F, 2 mM Tris, 0.1 M KCl, 2 mM $MgCl_2$, 0.2 mM ATP 0.1 mM $CaCl_2$ buffer (pH 7.8); Ox.A., performic oxidized actin; Ar.A., trinitrophenylated actin; TNBS, trinitrobenzenesulfonate; SDS, sodium dodecylsulfate

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some of their antigenic determinants and their ability to interfere with actin polymerisation.

2. MATERIALS AND METHODS

Rabbit actin and its performic axidized derivatives were obtained as in [7,12]. F-actin was also labelled by ^{125}I using Bolton and Hunter reagent [13]. F-actin was trinitrophenylated on lysine residues [10] in 0.1 M sodium bicarbonate, 0.1 M KCl, 0.7 mM $MgCl_2$ buffer (pH 8.2) with 10 M excess reagent/actin for 15 min at 25°C. After exhaustive dialysis at 4°C, F-actin was pelleted by ultracentrifugation for 1.5 h at 160000 × g. The number of arylated lysine residues (3/mol actin) was determined from absorbance at 346 nm [10]. These modified actins were further purified by SDS-preparative PAGE, before sheep immunization [7]. Actin antibodies were isolated [7] and insolubilized on Magnogel 44 resin [14]. Fab fragments were derived from Ox.A. antibodies using a papain digestion [15] and purified on immobilized actin [7]. G-actin in 2 mM Tris, 0.2 mM ATP, 0.1 mM $CaCl_2$ buffer (pH 7.8) was partially cleaved by staphylococcal V_8 protease [16]. The hydrolysis was followed by SDS–PAGE [17]. Two major fragments of M_r about 26000 and 16000, respectively, appear simultaneously in good accord with an M_r of 42000 for actin. The M_r 26000 fragment was described as the N-part of actin [16]. The M_r 16000 fragment which carries the 374 cysteine residue labelled with fluorescent probe [18] (fig.4) can be ascribed to the C-terminal part of the protein (submitted). Actin concentration was determined spectrophotometrically [19] or by colorimetry [20].

Peptide transfer on nitrocellulose sheet was performed as in [21]. The peroxidase activity of the second antiserum was revealed using 4-chloro-1-naphtol as substrate. Actin polymerisation was followed spectrophotometrically at 232 nm on a cary model 219 spectrophotometer [22]. Turbidimetry experiments were performed as described in fig.2. The reaction was monitored at 350 nm. In some experiments, the mixture was filtered through 0.22 μm millipore membrane. The retention of F-actin on this kind of membrane was >80%.

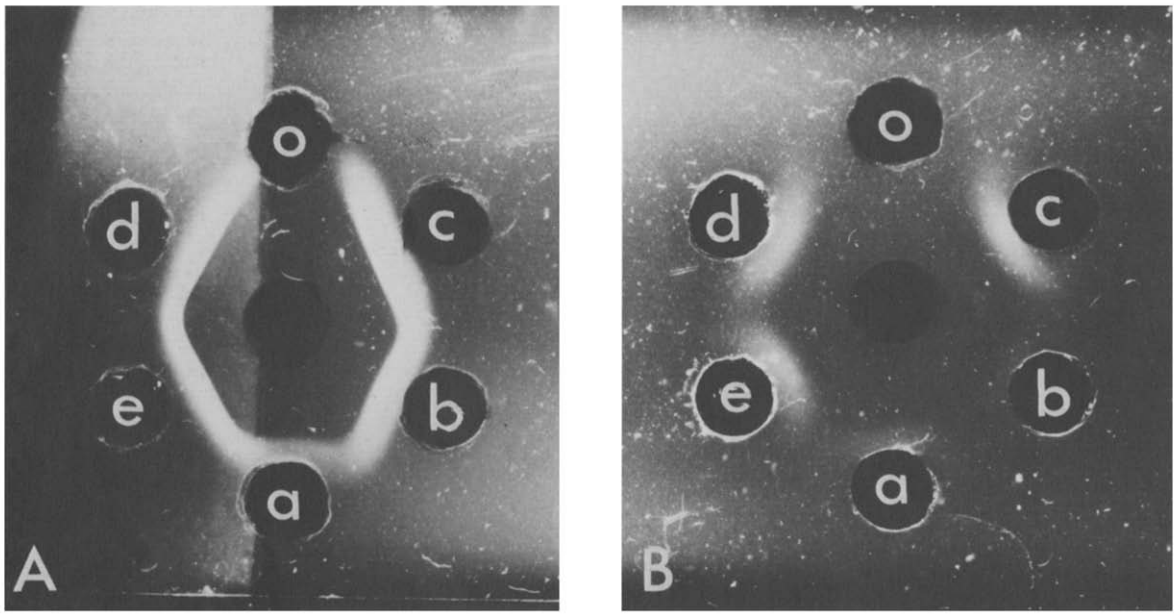

Fig.1. Immunodiffusion test of unfolded actin–anti-actin interactions: (A) Ox.A. antisera; (B) Ar.A. antisera; (O) preimmune sheep serum; (a–e) 1–5 month antisera, respectively; (center well) unpurified *S*-carboxymethylated actin (1.1 mg/ml).

3. RESULTS

The activity of antisera to Ox.A. and to Ar.A. was checked for the occurrence of precipitating antibodies against unfolded actin through immunodiffusion test (fig.1). The two lots of antisera are able to aggregate unfolded actin but not to the same extent. The immunoreactivity, strong for the Ox.A. antiserum, increased along the immunization process for Ar.A. antiserum. In both cases, the reaction against unfolded actin was specific.

The reactivity of the two corresponding purified antibodies was then tested against native F-actin using two independant analytical methods. Turbidimetry measurements of antibody–F-actin interactions seem to evidence a very different behaviour of the two lots of antibodies. The 'in vitro' binding of Ar.A. antibodies leads to the formation of insoluble aggregates while no effect apparently occurs with the other antibodies even after 2 h incubation (fig.2).

Through the ratio-immunoassay, it appears (fig.3) that the two lots of insolubilized antibodies are able to interact with F-actin but not to the same extent as compared to their reactivity towards unfolded actin. It can be evaluated that about 20% of Ox.A. antibodies can react with F-actin instead of about 70% of Ar.A. antibodies. Moreover, to substantiate the specificity of the two kinds of antisera, the purified anti-actin antibodies were tested using nitrocellulose replicates of electrophoretic

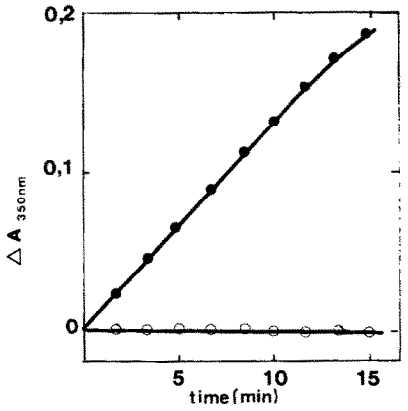

Fig.2. Spectral detection at 350 nm of insoluble F-actin–antibody complex formations. Actin (1 mg/ml) was incubated with (0.7 mg/ml) purified Ox.A. (○) or Ar.A. (●) antibodies in buffer F.

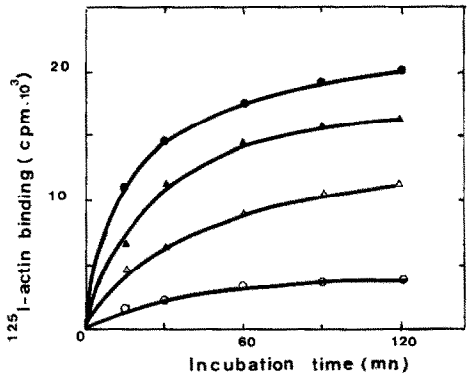

Fig.3. Radio-immunoassay analysis of interactions between insolubilized Ox.A. antibodies (●,○) or Ar.A. antibodies (▲, △) with 50 μg (75 000 cpm) of labelled, unfolded (●, ▲) or filamentous (○, △) actin, in 1 ml buffer F. At various times, aliquots (50 μl) of suspension were taken, washed as in [14] and counted for radioactivity; total bound radioactivity was plotted *vs* incubation time.

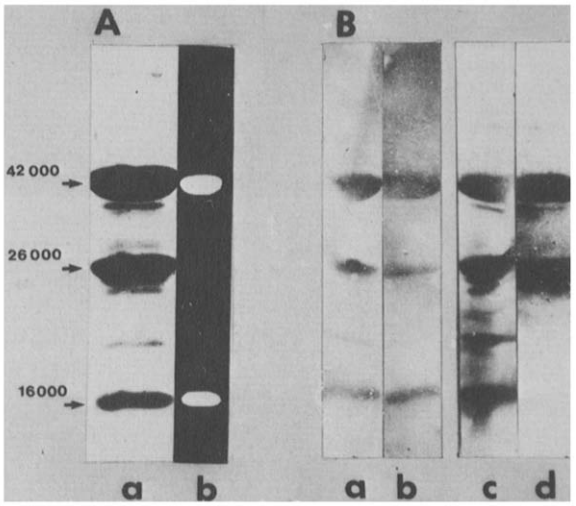

Fig.4. Antigenic reactivity of electrophoretic replicates from actin proteolytic digest. (A) SDS–12.5% polyacrylamide slab gel: (a) Coomassie blue coloration; (b) detection of fluorescent fragments derived from digestion of actin sample labelled in its Cys 374 by *N*-iodoacetyl-*N'*-(5-sulfonic-1-naphtyl) ethylene diamine. (B) Electrophoretic replicates: (a) reaction with Ox.A. antibodies; (b) reaction with Ox.A. antibodies not retained by F-actin; (c) reaction with Ar.A. antibodies; (d) reaction with Ar.A. antibodies not retained by F-actin.

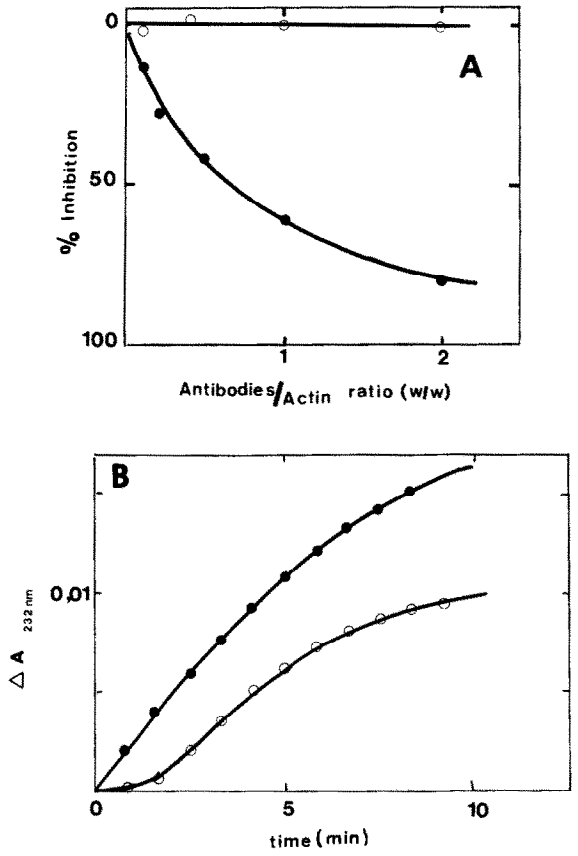

Fig.5. Effect of antibodies on actin polymerisation followed at 232 nm. (A) Inhibition (%) is plotted *vs* antibody/actin ratio (w/w); (●) Ox.A. antibodies; (○), Ar.A. antibodies. (B) Effect of Fab fragments derived from Ox.A. antibodies on actin polymerisation; actin (0.30 mg/ml) (●) alone or (○) incubated with Fab fragments (0.26 mg/ml).

patterns from peptidase hydrolysed actin. It appears (fig.4) through this analytical test that the two lots of antibodies similarly react with actin and its fragments. In addition, to compare their behaviour against filamentous actin, antibodies were incubated with F-actin. The unreacted antibodies were isolated by filtration (section 2) and analysed as above. The reaction of filtered Ox.A. antibodies is almost similar to that of starting antibodies (fig.4). In contrast, Ar.A. antibodies are drastically deprived of reactivity against the M_r 16000 fragment.

The ability of antibodies to prevent actin polymerisation has also been examined. Polymerisation was followed by measuring the change in the characteristic difference spectrum at 232 nm. In the concentration range used, we do not observe any interference of Ar.A. antibodies on actin polymerisation process (fig.5). Furthermore, this result indicates that in our experimental conditions, non-eventual interaction of IgG with actin [3] alters polymerisation. However, Ox.A. antibodies strongly decrease the polymerisation rate in connection with antibodies/G-actin ratio used. Lastly, Fab fragments derived from Ox.A. antibodies give a similar result (fig.5) underlining that this specific inhibition of polymerisation process is not directly linked with steric long-range interferences.

4. DISCUSSION

Ox.A. antibodies were able to decorate microfilaments and stress fibers [23,24] despite a weak reaction against polymerised actin [7,14]. However, they crossreact substantially against monomeric actin [2]. In contrast, natural auto-immune [5,9] or aggregated actin-induced [1,8] antibodies efficiently localize microfilaments but do not recognize G-actin [1,5].

From the present results it appears that the chemical treatment used to break natural tolerance against actin has a strong effect upon the specificity of produced antibodies. So oxidized actin, modified in 25 residues along the chain [7] and treated by SDS induces antibodies which crossreact weakly with F-actin and therefore are unable to precipitate it. Antigenic determinants preserved on F-actin do not appear exclusively born by the N- or C-terminal part of the protein.

Moreover, it appears that among the antigenic sites which are accessible on monomeric actin [2], some of them would be connected with either conformational changes [19] or actin–actin interaction which occurs during microfilaments formation. In contrast, arylated actin was only modified in 3 lysine residues which would be carried by the C-terminal part [11] of molecule. It induces antisera which weakly precipitate unfolded actin. The crossreaction with F-actin is more effective and reaches about 70%, leading to formation of insoluble complexes. In addition it is interesting to note a preferential location of antigenic determinants on the C-terminal part of F-actin.

The unfolded modified actins induce antibodies which are able to recognize unfolded, monomeric or filamentous actins and to interfere with actin polymerisation. The kind of chemical modifications performed affords to modulate the extent of these effects and to affect the location of antigenic determinants along the polypeptide chain.

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REFERENCES

[1] Dosseto, M. and Gorodis, C. (1980) Mol. Immunol. 17, 1219–1230.
[2] Benyamin, Y., Roger, M. and Robin, Y. (1981) CR Soc. Biol. 179, 462–467.
[3] Utter, G., Thorstensson, R., Norberg, R. and Fagraeus, A. (1982) Cell. Mol. Biol. 28, 97–101.
[4] Bulinski, J.C., Duman, S., Titani, K. and Hauschka, S. (1983) Proc. Natl. Acad. Sci. USA 80, 1506–1510.
[5] Andersen, P., Small, J.V., Andersen, H.K. and Sobieszek, A. (1979) Immunology 37, 705–709.
[6] Lazarides, E. and Weber, K. (1974) Proc. Natl. Acad. Sci. USA 71, 2268–2272.
[7] Benyamin, Y., Roger, M., Gabrion, J., Robin, Y. and Thoaï, N.V. (1979) FEBS Lett. 102, 69–74.
[8] Jockusch, B.M., Kelley, K.H., Meyer, R.K. and Burger, M.M. (1978) Histochemistry 55, 177–184.
[9] Fagraeus, A. and Norberg, R. (1978) Curr. Topics Microbiol. Immunol. 82, 1–13.
[10] Mühlrad, A. (1968) Biochim. Biophys. Acta 162, 444–451.
[11] Chen Lu, R. and Szilagyi, L. (1981) Biochemistry 20, 5914–5919.
[12] Spudich, J.A. and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871.
[13] Bolton, A.E. and Hunter, W.M. (1973) Biochem. J. 133, 529–539.
[14] Benyamin, Y., Roger, M., Robin, Y. and Thoaï, N.V. (1980) FEBS Lett. 110, 327–329.
[15] Edelman, G.M. and Marchalonis, J.J. (1967) in: Methods in Immunology and Immunochemistry (Williams, C.A. and Chase, M.W. eds) vol. 1, pp. 418–420, Academic Press, New York.
[16] Johnson, P., Wester, P.J. and Hikida, R.S. (1979) Biochim. Biophys. Acta 578, 253–257.
[17] Laemmli, U.K. (1970) Nature 227, 680–685.
[18] Takashi, R. (1979) Biochemistry 18, 5164–5169.
[19] Rich, S.A. and Estes, J.E. (1976) J. Mol. Biol. 104, 777–792.
[20] Bradford, M.M. (1976) Anal. Biochem. 72, 248–256.
[21] Yin, H.L., Albrecht, J. and Fattoum, A., (1981) J. Cell. Biol. 91, 901–906.
[22] Fattoum, A., Roustan, C., Reinberg, J. and Pradel, L.A. (1980) FEBS Lett. 118, 237–240.
[23] Gabrion, J., Travers, F., Benyamin, Y., Sentein, P. and Thoaï, N.V. (1980) Cell. Biol. Int. Rep. 4, 59–68.
[24] Pautrat, G., Benkoel, L., Benyamin, Y. and Thoaï, N.V. (1980) Cell. Biol. Int. Rep. 4, 881–887.