IMMUNOLOGICAL COMPARISON OF MUSCLE ACTINS FROM MAMMAL, BIRD AND FISH

A quantitative approach

Yves BENYAMIN, Michel ROGER, Jacqueline GABRION*, Yvonne ROBIN+ and Nguyen VAN THOAI

*Centre de Recherches de Biochimie Macromoléculaire du CNRS, BP 5051, 34033 Montpellier Cedex, *Laboratoire d'Histologie de la Faculté de Médecine, 2 Rue de l'Ecole de Médecine, 34000 Montpellier* and *+Laboratoire de Biochimie Marine de l'Ecole Pratique des Hautes Etudes, Collège de France, Place Marcelin-Berthelot, 75231 Paris Cedex 05, France*

Received 26 February 1979
Revised version received 5 April 1979

1. Introduction

Although antibodies against actin have been experimentally produced in animals in recent years [1], a major handicap in the immunochemical approach to actin is the poor immunogenicity of this antigen. Its highly conservative sequence has necessitated modification such as unfolding [2], insolubilization [3] or chemical linkage to particles [3] of this protein to promote immune responsiveness, but the antibody titers remained low [4] and the response uncertain [1]. Another difficulty is the apparent non-discriminating properties of most antisera to different sources of actin [1] which did not reflect the amino acid substitutions found by sequence analysis [5].

Here we describe an efficient method for producing anti-actin antibodies with a relatively high titer. We also present a quantitative study of the antigenic reactivity of unfolded and polymerised actins from rabbit and dog-fish striated muscles as well as from chicken gizzard.

2. Materials and methods

Rabbit, dog-fish and chicken actins were purified from acetone powders [6]. Their homogeneities were checked by SDS–acrylamide electrophoresis [7]. Viscosity determinations were done at 2°C using a Fica Viscosimatic MS viscosimeter. Actin concentrations were measured by its at A_{280} [8] or using the Lowry method [9] for unfolded forms. Rabbit performic acid-oxidized actin was obtained and analyzed as in [10]. This oxidized-actin was further purified by SDS–acrylamide preparative electrophoresis using the Buchler Poly-Prep 100 Apparatus under analytic conditions [7]. Actin was *S*-carboxymethylated in 8 M urea with iodoacetic acid [11]. Antiserum to oxidized rabbit actin was induced in sheep as follows: 4 mg protein was emulsified in 1.5 ml complete Freund's adjuvant (Difco) and injected by multisite intradermic procedure in the neck of the animal. Booster injections were given intramuscularly 1 month after the first injection and every 2 weeks, using the same quantity of emulsified antigen. Blood (500 ml) was taken 8 days after every booster injection and the antibody titers tested by immunodiffusion [12] against *S*-carboxymethylated actin. Anti-actin antibodies were isolated [13] from the sixth month antiserum on a rabbit *S*-carboxymethylated actin Sepharose 4B immunoadsorbant column.

Immunofluorescence localization was obtained by the indirect method [14]. Antigenic reactivity of actins was checked using a magnetic solid phase enzyme immunoassay [15] with alkaline phosphatase from *E. coli* (Sigma) as the enzymatic marker.

Address correspondence and reprints requests to: BP 5051, 34033 Montpellier, France

3. Results and discussion

The amino acid composition of the performic acid-oxidized actin is given in table 1 together with that of native rabbit muscle actin [16] This result shows that cysteine, methionine and tryptophan residues are almost quantitatively oxidized, the other residues being unaffected Electrophoretic analysis (fig 1) revealed that no contamination by degradation or polymerisation products remained in repurified oxidized-actin before immunization

The specificity and the enhancement of the immune response between the first and the sixth month is illustrated in fig 2 Only one precipitating arc is observed between non purified *S*-carboxymethylated actin and the two antisera The high antibody titer of the sixth month antiserum was confirmed after adsorption on immunoadsorbant ~150 mg purified antibodies were isolated from 50 ml antiserum Their activity was checked by immunodiffusion (fig 2) against unfolded actin and by indirect fluorescence on myofibrils in rat skeletal muscle sections (fig 3) No secondary precipitation arc or spurs with first or sixth month antisera is observed and the fluorescence is localized in the I-band It appears from these results that the oxidation of sulphur residues and tryptophans had strongly enhanced the immunogenic capacity of

Table 1
Amino acid composition of rabbit performic acid-oxidized actin after 24 h hydrolysis

Amino acid	Amino acid composition (res /mol)	
	Native actin	Oxidized actin
Aspartic acid	34	35 8
Threonine	28	27 9
Serine	22	22 2
Glutamic acid	39	40 9
Proline	19	17 8
Glycine	28	28
Alanine	29	27 9
Methionine	16	traces
Methionine sulfone	0	14 8
Isoleucine	29	27 6
Leucine	26	24 5
Tyrosine	16	15 3
Phenylalanine	12	12 8
Histidine	9	8 4
Lysine	19	18
Arginine	18	17 3
Tryptophan	4	0
Cysteine	5	0
Cysteic acid	0	4 8
Cystine	0	0

Fig 1 Electrophoretic analysis on SDS–polyacrylamide gel of repurified performic acid-oxidized actin (50 μg) from rabbit striated muscle

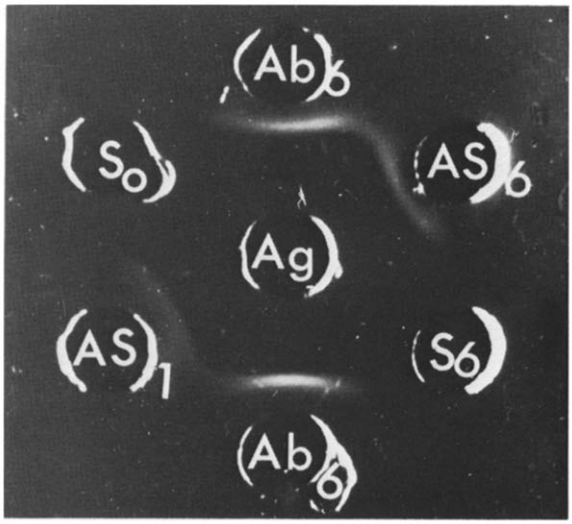

Fig.2. Immunodiffusion test of actin–anti-actin interactions after 48 h. (S_0), preimmune sheep serum; $(AS)_1$ first month antiserum; $(AS)_6$, sixth month antiserum; $(S)_6$, sixth month antiserum adsorbed on *S*-carboxymethylated actin–Sepharose 4B immunoadsorbant column and concentrated (10 ×) by ultrafiltration; $(Ab)_6$, isolated antibodies (3 mg/ml) from sixth month antiserum; (Ag), non-purified *S*-carboxymethylated actin (1 mg/ml) from rabbit skeletal muscle.

rabbit muscle actin. A similar conclusion was drawn of creatine kinase from an immunochemical study [10]. The reactivity of antibodies to filamentous actin observed by indirect fluorescence on myofibrils revealed that antibodies to accessible determinants on the thin fir/aments have been elicited, but the amplitude of this crossreactivity was not appreciable.

The antigenic crossreactivity of unfolded and polymerized rabbit actins checked by enzyme-immunoassay is illustrated in fig.4. In 0.1 M KCl

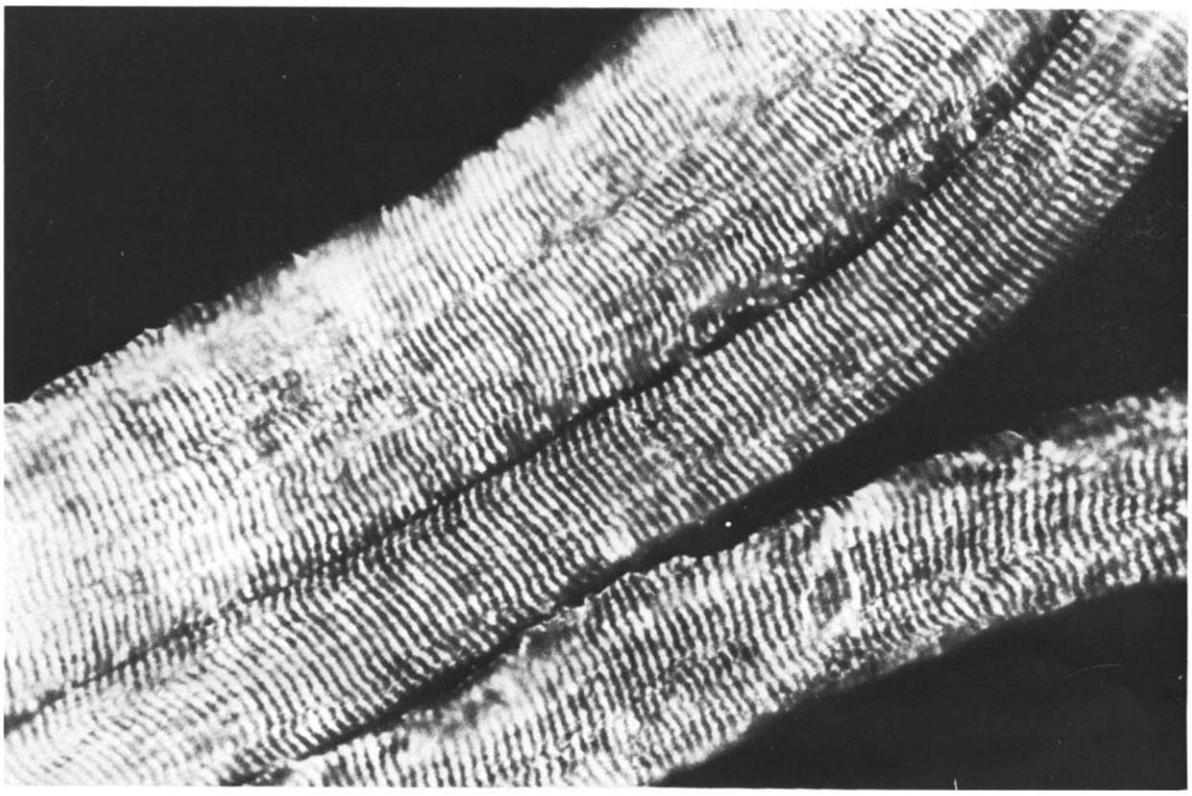

Fig.3. Immunofluorescent staining of fresh frozen sections from rat skeletal muscle using purified sheep anti-oxidized actin antibodies (20 μg/ml) and FITC rabbit anti-sheep IgG.

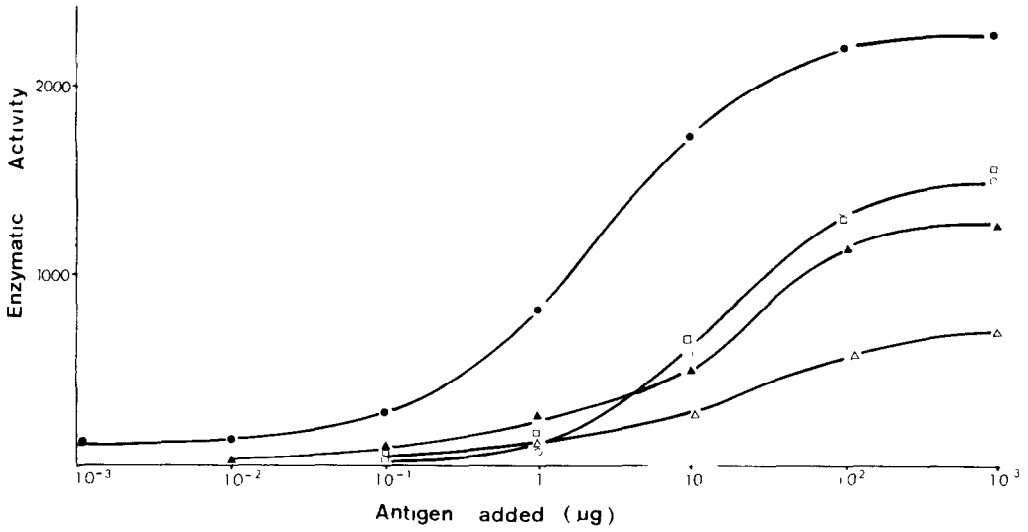

Fig 4 Quantitation of antigenic reactivity of unfolded *S*-carboxymethylated actin in 0 1 M KCL (●–●) or 2 mM $MgCl_2$ (▲–▲) and polymerized actin in 0 1 M KCl (○–○), 0 1 M KCl + 2 mM $MgCl_2$ (□–□) or 2 mM $MgCl_2$ (△–△) Antigens were incubated, 2 h at 20 C, with 50 µl insolubilized Ab (25 µg) in 1 ml 2 mM Tris, 0 2 mM ATP, 0 1 mM $CaCl_2$, 1% BSA (pH 7 4) buffer supplemented by salts used for polymerization Particles were washed 6 times with the same buffer and reincubated in the same conditions with 20 µl (40 µg) of alkaline phosphatase–antibodies (2 1, w/w) glutaraldehyde copolymerized After 6 washings as above, the solid phase was supplemented with 1 ml 2 mM *p*-nitrophenylphosphate in 1 M Tris - HCl (pH 8) and incubated for 30 min at 20°C The reaction was stopped with 100 µl 10 N NaOH and A_{410} read The results are expressed in pmol hydrolyzed substrates min^{-1}

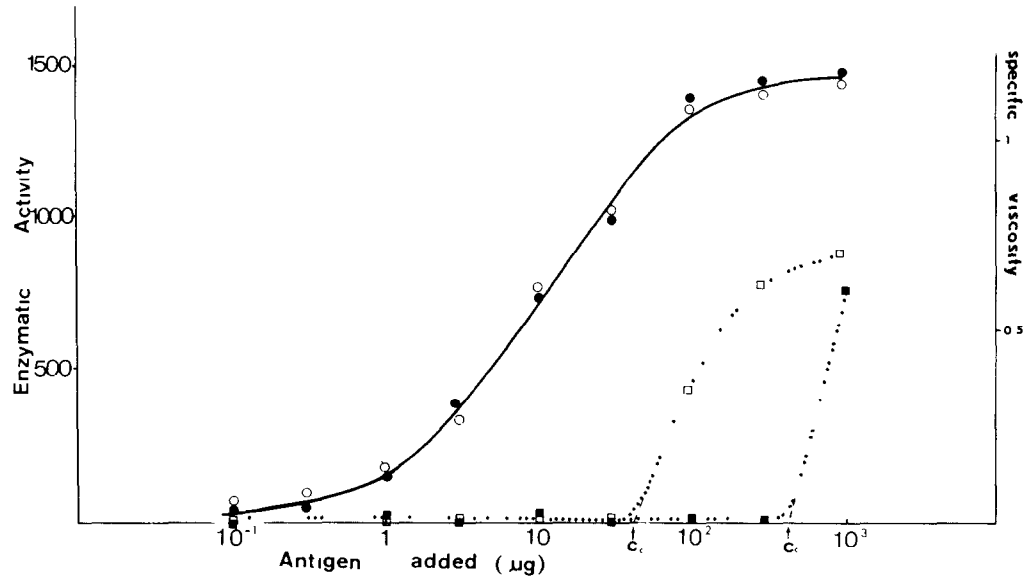

Fig 5 Quantitation of changes in antigenic reactivity of native actin with temperature Increasing G-actin concentrations in 0 1 M KCl buffer were incubated 24 h at 20°C (○–○) or at 2°C (■–■), their specific viscosities and the critical concentrations (Cc) being evaluated at 2°C Aliquots for immunoassays were removed and incubated at 20°C (○–○) or 2°C (●–●) as in fig 4 with incubation times of 12 h to take account of temperature effect on immune interactions

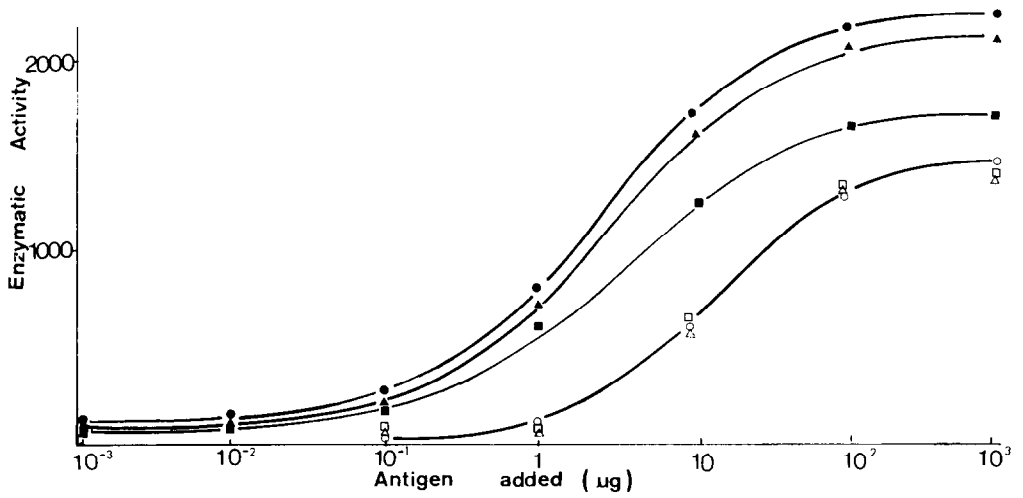

Fig.6. Immuno-crossreactivities of unfolded actins (full symbols) and 0.1 M KCl + 2 mM $MgCl_2$ polymerized actins (open symbols) of striated-muscle from rabbit (●—●, ○—○), dog-fish (▲—▲, △—△) and smooth muscle from chicken gizzard (■—■, □—□). Immunoassay conditions were as described in the legend to fig.4.

buffer, a sigmoid curve is obtained between 0.01 µg and 1000 µg for unfolded actin with a roughly linear part between 0.1 µg and 100 µg. In F-actin assay the response is shifted towards higher antigen concentration and its amplitude is about 65% of that of the unfolded actin. This result is unmodified when polymerization process is induced by 0.1 M KCl supplemented by 2 mM $MgCl_2$. At low ionic strength in 2 mM $MgCl_2$ (fig.4) a loss of dose responsiveness is observed for unfolded and filamentous actins, but their crossreactivity is similar to that observed at higher ionic strength. On the other hand when the critical concentration needed for actin polymerization [17] is shifted [8] from about 40 µg at 20°C to about 400 µg at 2°C (fig.5), no change is observed in the reactivity curve of actin to its antibodies. It appears therefore that rabbit muscle F-actin polymerized in different conditions retains, towards anti-unfolded actin antibodies, 65% of the reactivity of the polypeptidic chain and confirms quantitatively its ability to fix antibodies on the I-band of myofibrils. Moreover native actin which has been described as a monomeric form in 0.1 M KCl below the critical concentration [17], has the same reactivity as does the filamentous form, it is possible that polymerization sites were not immunogenic in performic acid-oxidized actin.

The reactivity of anti-rabbit oxidized actin antibodies to other muscle actins is illustrated in fig.6. It appears that the filamentous actins from smooth and striated muscles from bird, cartilagenous fish and mammal are not differentiated, but the unfolded forms cross-react with rabbit muscle actin to the extent of 75% for gizzard actin and 95% for dog-fish muscle actin. This result could reflect the small differences in amino acid sequences of actins from chicken gizzard [5], trout striated muscle [18] and rabbit skeletal muscle [16].

Acknowledgements

The authors are grateful to Martine Mesquida and Jacques Martin for their expert technical assistance. This work was supported by a grant from DGRST (AC 76 7 1185).

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