Definition of a Ca^{2+} -sensitive interface in the plasma gelsolin–actin complex

Ahmed HOUMEIDA, Veronique HANIN, Jeanne FEINBERG, Yves BENYAMIN and Claude ROUSTAN*

UPR 8402 Centre de Recherches de Biochimie Macromoléculaire CNRS, U249 INSERM, Laboratoire de Biochimie et Ecologie des Invertébrés Marins (EPHE), Université de Montpellier I, Route de Mende, BP 5051, 34033 Montpellier Cedex, France

Gelsolin is a Ca^{2+} -dependent protein which severs actin filaments, caps their fast-growing ends and promotes nucleation. We report here results that delimit one of the interfaces between serum gelsolin and actin monomer. An actin-derived synthetic peptide (amino acids 305–326 of actin) coupled to a hydrophilic resin was tested for its possible interaction with gelsolin. We selected this sequence because it corresponds to a region implicated in the gelsolin–actin complex in a previous work [Boyer, Feinberg, Hue, Capony, Benyamin & Roustan (1987) *Biochem. J.* **248**, 359–364]. We showed that this actin sequence is located at the surface of the actin molecule and observed a Ca^{2+} -sensitive binding of gelsolin to this actin-derived peptide. In addition, by using a chymotryptic digest of gelsolin, we reported that only the C-terminal half of gelsolin interacts with the actin-(305–326)-peptide. These results suggest that the Ca^{2+} -sensitive interface includes both amino acids 305–326 of actin and probably amino acids 660–738 of gelsolin.

INTRODUCTION

Gelsolin is a 82 kDa monomeric protein found in the blood plasma and many types of cells in the tissues of vertebrates (Kwiatkowski *et al.*, 1986). This protein appears to regulate the length of actin filaments, and its activity is modulated by Ca^{2+} and polyphosphoinositides (Yin, 1988). Therefore, in plasma, gelsolin is thought to function, in conjunction with vitamin D-binding protein, in the elimination of circulating actin (Janmey *et al.*, 1986; Méjean *et al.*, 1987a; Kwiatkowski *et al.*, 1988).

Extensive investigations have established that gelsolin is a multifunctional protein in that it nucleates polymerization of actin, binds to the barbed end of actin filaments and severs actin filaments. Hence several actin-binding domains are expected to be present. At least three interface regions have been identified by using proteolytic cleavage or segmental deletion mutagenesis (Bryan, 1988; Way *et al.*, 1989). The stoichiometry of the interaction is one gelsolin molecule for two actin monomers (Porte & Harricane, 1986; Harris, 1988). The occurrence of a strong tandem repeat in the amino acid sequence suggests a gene duplication dividing the gelsolin molecule into two roughly equal domains, i.e. C- and N-terminal-half regions (Kwiatkowski *et al.*, 1986). Analysis of the amino acid sequence also reveals six large segment repeats, each of about 15000 Da, numbered 1–6 starting from the N-terminal end (Way & Weeds, 1988). This explains the proteolytic pattern obtained with several different proteinases (Chaponnier *et al.*, 1986; Way & Weeds, 1988).

To elucidate these multifunctional properties, several authors (Kwiatkowski *et al.*, 1989; Way *et al.*, 1989) have studied the individual roles of the isolated segments or the deleted proteins obtained by mutagenesis. In fact, the N-terminal segment (segment no. 1; amino acids 1–150) possesses the high-affinity Ca^{2+} -independent actin-binding site. Gelsolin segments 2–3 (amino acids 151–406) bind to another actin monomer in a Ca^{2+} -independent manner, but the interaction of actin with this site in the intact gelsolin molecule is Ca^{2+} -sensitive.

Finally, the large fragment (segments 4–6; amino acids 407–755) contained a single Ca^{2+} -dependent site which interacts

with the first actin molecule (Way *et al.*, 1989). In addition, the presence of the three binding domains is supported by cross-linking experiments (Sutoh & Yin, 1989).

Although the interaction between gelsolin and actin has been extensively investigated, relatively little information is available on the actin sequences actually involved in these interfaces. Cross-linking experiments (Doi *et al.*, 1987; Sutoh & Yin, 1989) showed a possible proximity between gelsolin and the subdomain I of actin, which, as defined by X-ray crystallography, contains the N-terminal and C-terminal extremities (Kabsch *et al.*, 1990). We have also implicated some regions of subdomains III and I of actin in the contact sites with gelsolin. These regions are located near or at specific antibody epitopes (around Met-305, Met-325 and Met-355) (Boyer *et al.*, 1987).

The importance of the C-terminal part of actin for the interaction is also substantiated by the observation that vitamin D-binding protein, which is known to interact near the profilin site at amino acids 226–375 in the sequence of actin (Goldsmith-Clermont *et al.*, 1986), competes with gelsolin for binding to the actin monomer (Coue *et al.*, 1986).

In the present paper, we report the localization of a binding site of gelsolin in the C-terminal part of actin. By using a synthetic peptide, actin-(305–326), we demonstrated the presence of an interface including this sequence and the domain of gelsolin containing segments 4–6.

MATERIALS AND METHODS

Proteins and derivatives

Rabbit skeletal muscle, bovine cardiac muscle and scallop muscle actins were obtained from acetone-dried powders (Spudich & Watt, 1971). Bovine aortic actin (Cavadore *et al.*, 1985) was a gift from J. C. Cavadore, Centre de Recherches de Biochimie Macromoléculaire, Montpellier, France. S-Carboxymethylated actin was produced as previously described (Hirs, 1967). The actin fragment containing amino acids 285–375 was purified by electroelution from SDS slab gels after cleavage at

Abbreviations used: Boc, t-butyloxycarbonyl; NBD, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole.

* To whom correspondence should be addressed at: Centre de Recherches de Biochimie Macromoléculaire, Route de Mende, BP 5051, F-34033 Montpellier Cedex, France.

cysteine residues by 2-nitro-5-thiocyanobenzoic acid (Boyer *et al.*, 1985).

Bovine plasma gelsolin was purified as described by Souza *et al.* (1985). Its digestion by chymotrypsin (gelsolin/chymotrypsin ratio 100:1, w/w) was conducted for a relatively long time (90 min) (Bryan, 1988) to eliminate the CT41 fragment, which is hydrolysed into CT15 and CT28 fragments. Purification of CT15 and CT47 was carried out by h.p.l.c. using DEAE resin as described by Bryan (1988). The samples were salted out on a C4 Aquapore butyl (2 mm $\times$ 30 mm) resin column (Brownlee), and their *N*-terminal sequences were determined on an Applied Biosystems model 470 A gas phase sequence coupled to an on-line phenylthiohydantoin analyser model 120 A.

Antibodies to peptidyl-resin were elicited in rabbit by direct injection of the crushed peptidyl-resin emulsified in Freund's adjuvant as described previously (Hanin *et al.*, 1989; Hanin, 1990). The antibodies were purified by affinity chromatography on an *S*-carboxymethylated actin column (Andersson *et al.*, 1978). Antibodies to serum gelsolin were also produced in rabbit as described by Souza *et al.* (1985). Anti-IgG antibodies labelled with alkaline phosphatase were purchased from Biosys (Compiègne, France).

Solid-phase synthesis of the fragment of skeletal muscle actin containing amino acids 305–326

The actin fragment was synthesized on a polyacrylamide-based support (Expansin; Expansia, Aramon, France) which is a co-polymer of *N*-acryloylpyrrolidine and *N*-acryloyl- β -alanine methyl ester reticulated using ethylenebisacrylamide. The polymerization procedure has been previously described in detail (Calas *et al.*, 1985). After polymerization, the resin was derivatized with ethylenediamine (48 h, room temperature). The final resin contained 0.6 mmol of amine groups/g. All operations of solid-phase peptide synthesis were carried out as previously described (Hanin *et al.*, 1989), under mechanical shaking using a 50 ml glass reactor. The *t*-butyloxycarbonyl (Boc)-protected amino acid derivatives (symmetrical anhydrides) were incorporated successively using a previously reported synthesis protocol (Castro *et al.*, 1975). The completion of the reaction was monitored by ninhydrin assay or chloranil assay for proline residues on a peptidyl-resin sample. Trifluoroacetic acid (30 % in dichloromethane) was used to remove Boc. Side-chain-protecting groups were 2-chlorobenzoyloxycarbonyl for Lys, *O*-Bzl (*O*-benzyl) for Thr, Ser, Glu and Asp, Tos (tosyl) for Arg and Dcb (2,6-dichlorobenzyl) for Tyr. They were removed following synthesis by treatment with trifluoromethanesulphonic acid (1 M in trifluoroacetic acid) in the presence of anisole, metacresol and butanedithiol, for 2 $\times$ 30 min at 0 °C. The amount of peptide linked to the resin and the amino acid composition were determined by amino acid analysis after hydrolysis of the peptidyl-resin for 48 h in 6 M-HCl containing 6 mM-phenol.

Analytical methods

SDS/polyacrylamide slab gel electrophoresis was run according to the procedure of Laemmli (1970). E.l.i.s.a. was used to monitor interactions between actin, actin fragment and gelsolin or antibodies. Details of the method have been reported in previous papers (Méjean *et al.*, 1987a,b). Specific indications are given in Figure legends. In the particular case of e.l.i.s.a. with actin-(305–326), the peptidyl-resin previously crushed was added to the wells of microtitre plates (10 μ g of peptide/well) (Hanin *et al.*, 1989) and then treated as above.

Affinity chromatography with peptidyl-resin was carried out as follows. Crushed resin was used with an equivalent volume of Bio-gel P6 resin and equilibrated with 2 mM- CaCl_2 /3 mM-KCl/140 mM-NaCl/50 mM-Tris buffer, pH 7.8 (buffer A) or with

140 mM-NaCl/10 mM-phosphate/2.5 mM-KCl, pH 7.5 (buffer B). The material was eluted from the column either by buffer A supplemented with 10 mM-EGTA when the column was equilibrated with buffer A, or by 10 % dioxan/20 mM-phosphate buffer, pH 12 (buffer C) when the column was equilibrated with buffer B. In the latter case the eluate was neutralized. The absorbance was monitored at 280 nm.

RESULTS

Interaction of plasma gelsolin with various actins and actin fragments

Interaction of gelsolin with various isoactins coated on to microtitre plates was tested by using anti-gelsolin antiserum. The results presented in Fig. 1 show that gelsolin interacted similarly with the four isoactins examined (rabbit skeletal, bovine aortic, bovine cardiac and scallop adductor muscle actins). Therefore gelsolin appears to bind to sequences in actin that are conserved during evolution.

Fig. 1. Interaction of serum gelsolin with various isoactins monitored by e.l.i.s.a.

Gelsolin was incubated in 3 mM-KCl/140 mM-NaCl/50 mM-Tris buffer, pH 7.8, containing 4 mM- CaCl_2 and 0.2 mM-ATP in the presence of coated G-actin. ○, Rabbit skeletal muscle actin; ●, bovine aortic actin; □, bovine cardiac actin; ■, scallop adductor muscle actin. After incubation with purified anti-gelsolin antibodies (0.15 μ g/ml) and then with anti-(rabbit IgG) antibodies labelled with alkaline phosphatase, the gelsolin interaction was monitored at 405 nm.

Fig. 2. Effect of Ca^{2+} on the interaction of serum gelsolin with coated G-actin monitored by e.l.i.s.a.

Gelsolin was incubated in 3 mM-KCl/140 mM-NaCl/50 mM-Tris buffer, pH 7.8, containing either 4 mM- CaCl_2 /0.2 mM-ATP (●) or 10 mM-EGTA (○) in the presence of coated skeletal muscle G-actin. The gelsolin interaction was monitored at 405 nm.

Fig. 3. Interaction of serum gelsolin with the C-terminal fragment (amino acids 285-375) of actin monitored by e.l.s.a.

Gelsolin was incubated in 3 mM-KCl/140 mM-NaCl/50 mM-Tris buffer, pH 7.8, containing either 4 mM- CaCl_2 /0.2 mM-ATP (●) or 10 mM-EGTA (○) in the presence of coated actin fragment. The interaction was monitored at 405 nm.

Table 1. Sequence and amino acid composition of the actin-(305-326) synthetic fragment derived from skeletal muscle actin

The sequence was: Met-Tyr-Pro-Gly-Thr-Ala-Asp-Arg-Met-Gln-Lys-Glu-Ile-Thr-Ala-Leu-Ala-Pro-Ser-Thr-Met-Lys.

Amino acid	Composition (mol of residue/ mol of fragment)	
	Found	Calculated*
Asp	0.92	1
Thr	2.97	3
Ser	0.69	1
Glu	2.04	2
Pro	1.70	2
Gly	0.98	1
Ala	3.27	3
Met	2.82	3
Ile	1.09	1
Leu	1.21	1
Tyr	0.68	1
Lys	1.84	2
Arg	1.07	1

* The calculated values are based on the known sequence of skeletal muscle actin from amino acid 305 to 326.

The Ca^{2+} -dependence of the interaction with coated actin was also studied. The binding of gelsolin to actin in the presence of 2 mM free Ca^{2+} was abolished by excess EGTA when the latter was added to the reaction mixture before interaction (Fig. 2). This result suggests that binding of gelsolin to actin in both solution and heterogeneous phase is Ca^{2+} -dependent and that, in our experimental conditions, the possibility of a non-specific absorption is excluded.

For the precise localization of gelsolin-binding sites in the C-terminal region of actin (Boyer *et al.*, 1987), experiments were carried out with the actin fragment (amino acids 285-375) derived from chemical cleavage of actin at cysteinyl residues (Boyer *et al.*, 1985). Fig. 3 shows the pattern of interaction between gelsolin and this actin fragment. The reactivity of gelsolin with the fragment was of about the same order of magnitude as with monomeric actin and was also Ca^{2+} -dependent.

Fig. 4. Reactivity of anti-actin (305-326) antibodies

Purified antibodies were incubated with coated G-actin (□), synthetic peptide coupled to the resin (■) and the resin alone (○). The binding of the antibodies was monitored at 405 nm.

Fig. 5. Interaction of gelsolin with the synthetic peptide actin-(305-326) coupled to resin

(a) Gelsolin was incubated for 1 h in 3 mM-KCl/140 mM-NaCl/50 mM-Tris buffer, pH 7.8, containing either 4 mM- CaCl_2 /0.2 mM-ATP (●) or 10 mM-EGTA (○) in the presence of coated peptidyl-resin. (b) Gelsolin was incubated with coated peptidyl-resin for 1 h in the presence of CaCl_2 , followed by another 1 h in the presence of either 4 mM- CaCl_2 (●) or 10 mM-EGTA (○). Binding was monitored at 405 nm.

Involvement of amino acids 305-326 of actin in the interaction with gelsolin

The peptide corresponding to amino acids 305-326 of the actin sequence was synthesized on an acrylamide resin as described in the Materials and methods section. The yield of substitution of the peptide on the resin was measured and corresponded to 0.4 g of peptide/g of resin. The peptide showed an amino acid composition (Table 1) that was identical with that of the

Fig. 6. Location of one binding site in the C-terminal part of bovine serum gelsolin

(a) Binding of chymotryptic digest of gelsolin to peptidyl-resin. Lane 1 shows the molecular mass standards; lane 2 shows the material obtained after elution of the peptidyl-resin column by 10 mM-EGTA (see the Materials and methods section); lane 3 shows the pattern of a chymotryptic digest of gelsolin. Gels were stained by Coomassie Blue. (b) and (c) The fractions containing the purified CT47C fragment (b, lane 1) and CT15N fragment (c, lane 1) were passed through the peptidyl-resin affinity column and the retained material was eluted at alkaline pH (see the Materials and methods section) (lanes 2). Lane 3 in (b) shows an actin standard. Proteins were revealed by silver staining.

corresponding segment of actin. The accessibility of the peptide on the resin and of this sequence in the actin molecule was checked by direct e.l.i.s.a. using antibodies induced by the peptidyl-resin. Previously the antibodies were purified by affinity chromatography on an unfolded carboxymethylated actin column (see the Materials and methods section) to avoid antibody reactivity towards the resin itself in e.l.i.s.a. Fig. 4 shows a good reactivity of the antibodies to the coated peptide as well as to G-actin. The latter result demonstrated the accessibility of the sequence, or at least of a part of the sequence, in the native actin.

Peptidyl-resin was then used to characterize a possible binding region for gelsolin corresponding to a precise sequence in the monomeric actin. The interaction was studied by direct e.l.i.s.a. using anti-gelsolin antiserum (Fig. 5a) to give a saturation curve. In this case, the interaction appeared to be Ca^{2+} -dependent, as shown by the lack of binding when 10 mM-EGTA was added to the coated peptide before incubation with gelsolin. Control experiments with non-substituted resin showed only a very low level of interaction. On the other hand, addition of excess EGTA after the interaction of gelsolin in the presence of Ca^{2+} resulted in greatly decreased formation of the gelsolin-peptide complex (Fig. 5b).

Amino acids 305–326 of the actin monomer thus appear to be accessible to gelsolin binding and hence are implicated in the actin-gelsolin interface. We then attempted to determine the counterpart in the gelsolin molecule that is involved in the interface.

Determination of the interface between actin and gelsolin

Plasma gelsolin can be cleaved into three major fragments by chymotrypsin (Chaponnier *et al.*, 1986). In human plasma gelsolin, whose amino acid sequence is known (Kwiatkowski *et al.*, 1986), these fragments are the N-terminal CT15N (Ser-24–Phe-149), the C-terminal CT47C (Ala-407–Ala-755) and the middle fragment CT31N, spanning between CT15N and CT47C. The chymotryptic pattern obtained with bovine plasma gelsolin showed four fragments and some minor products (Fig. 6).

To determine the fragment of gelsolin that binds to actin peptide, the whole chymotryptic digest was first passed through an affinity column filled with the peptidyl-resin in buffer A or B (see the Materials and methods section). After washing, the material bound to the peptide was eluted either by 10 mM-EGTA or at pH 12 in 10% dioxan. The eluted material, subjected to SDS/PAGE (Fig. 6a), showed a major band with slower electrophoretic mobility than actin, corresponding to CT47C of the chymotryptic digest. In addition, some faint bands of higher molecular mass were also noticed after silver staining. To confirm the identity of the gelsolin fragment that binds to actin-(305–326), the peptides in the chymotryptic digest were purified by h.p.l.c. on a DEAE column as described in the Materials and methods section. The two major components obtained with sufficient purity were subjected to micro-sequencing and were identified by their N-terminal sequences as CT15N and CT47C. When these two fractions were passed through the peptidyl-resin affinity column, only the one corresponding to CT47C was retained (Figs. 6b and 6c).

DISCUSSION

Recent results describing the three-dimensional structure of the actin-DNAase I complex by X-ray analysis (Kabsch *et al.*, 1990) showed that the actin molecule can be divided in two lobes, a small lobe corresponding to subdomains I and II and a large lobe of subdomains III and IV, with a pronounced cleft in between.

Further, the N- and C-terminals are located in subdomain I in close proximity to each other (Boyer *et al.*, 1985; Kabsch *et al.*, 1990) and are accessible to interacting ligands. The C-terminus, spanning residues 300–375, is located opposite the DNAase I interface (subdomains II and IV). In fact, the region around residue 302, located in subdomain III, lies near the ADP site. Thus the fragment containing amino acids 300–375 appears to connect the two lobes of the actin molecule. Previous reports (Boyer *et al.*, 1987) showed a competition between specific antibodies raised against epitopes around Met-305, Met-326 and Met-355 and gelsolin for binding to actin, implicating this region in the actin-gelsolin interface. These results have prompted us to synthesize a small fragment of actin corresponding to amino acids 305–326 for more precise experimental analysis. This region, except residue 306, represents a highly conserved sequence of actin, and its hydrophilicity pattern, in accordance with recent crystallographic studies (Kabsch *et al.*, 1990), indicated a good probability for its presence being at the surface of the actin molecule, making it accessible for gelsolin binding.

The peptide was then synthesized on a polyacrylamide support because the latter, as opposed to styrene, is hydrophilic and can be used directly in e.l.i.s.a. or affinity chromatography (Hanin *et al.*, 1989). The use of small synthetic or natural peptides is an efficient tool with which to determine interfaces between proteins. With contractile proteins this method has proved to be particularly successful. For example, the interactions of the N-terminal sequence of actin (amino acids 1–44) with S-1 (Levine & Moir, 1985), of actin with a fragment around SH₁ of S-1 (Suzuki

et al., 1987) and of cofilin-(122–126) with actin (Yonezawa *et al.*, 1989) have all been studied by this approach. Peptidyl-resins can equally be used to produce antiserum (Hanin *et al.*, 1989). We have obtained a specific rabbit antiserum which has a similar reactivity towards both peptidyl-resin and G-actin (Fig. 4), demonstrating the accessibility of at least a part of the sequence containing residues 305–326 in the actin monomer. A direct interaction between gelsolin and this sequence has been shown, the binding being Ca^{2+} -dependent. The association of this part of actin with a Ca^{2+} -dependent site in gelsolin is suggested, in view of the presence of two Ca^{2+} -sensitive sites in domains 4–6 and 2–3 of the native gelsolin molecule (Way *et al.*, 1989). So, to determine accurately the domain of gelsolin involved, we have studied the interaction of cleaved gelsolin on an actin-(305–326) peptidyl-resin affinity column and shown that the same major fragment is released either by EGTA or at alkaline pH. The Ca^{2+} -dependence of binding of the gelsolin fragment suggested that the implicated fragment is the C-terminal half of gelsolin (domains 4–6) (Way *et al.*, 1989). This interpretation was supported by examination of the apparent molecular mass of the eluted fragment. Further, the purified CT47C peptide of gelsolin, identified by its N-terminal sequence, is able to react with actin-(305–326).

We conclude that a part of the region encompassing domains 4–6 of gelsolin, and more accurately amino acids 666–738 as judged by mutagenesis experiments (Kwiatkowski *et al.*, 1989), interacts with the sequence containing amino acids 305–326 of actin. Residues 666–738 in gelsolin cover essentially the sixth repeat domain and show some sequence similarity with other severing proteins. Indeed, severin shows sequence similarity to the C-terminal half of gelsolin, in particular between a repeat structure of amino acids 179–215 and 290–326 of severin and the gelsolin sequence 688–724 (André *et al.*, 1988).

It is also interesting to note that the interaction of the C-terminal part of gelsolin impeded actin polymerization (Way *et al.*, 1989). The first molecule of actin would interact with the C-terminal site located in domain 6 of gelsolin, then with the Ca^{2+} -independent site in domain I, giving a stable EGTA-independent complex. The interaction of actin-(666–738), unlike the N-terminal domain (domain I), does not affect the fluorescence of actin labelled with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD) at Lys-373 (Way *et al.*, 1989). Similarly, antibody directed to a part of the sequence 305–326 of actin does not alter the fluorescence of NBD (A. Houmeida, Y. Benyamin & C. Roustan, unpublished work). In addition, cross-linking experiments (Sutoh & Yin, 1989) suggested the proximity of some parts of gelsolin to the C-terminal extremity of actin. Therefore these results suggest the occurrence of another site of gelsolin (more probably domain I) at some spatial proximity to the Lys-373 of actin.

This work was supported by grants from CNRS-INSERM (no. 1223), the Association Française contre les Myopathies, and the Ministère de la Recherche et de l'Enseignement Supérieur. A.H. is the recipient of

a scholarship from the Ministère de l'Education Nationale de Mauritanie.

REFERENCES

- Andersson, K., Benyamin, Y., Douzou, P. & Balny, C. (1978) *J. Immunol. Methods* **23**, 17–21.
- André, E., Lottspeich, F., Schleicher, M. & Naegel, A. (1988) *J. Biol. Chem.* **263**, 722–727.
- Boyer, M., Roustan, C. & Benyamin, Y. (1985) *Biosci. Rep.* **5**, 39–46.
- Boyer, M., Feinberg, J., Hue, H. K., Capony, J. P., Benyamin, Y. & Roustan, C. (1987) *Biochem. J.* **248**, 359–364.
- Bryan, J. (1988) *J. Cell Biol.* **106**, 1553–1562.
- Calas, B., Mery, J., Parelo, J. & Cave, A. (1985) *Tetrahedron* **41**, 5331–5339.
- Castro, B., Dormoy, J. R., Evin, G. & Salve, C. (1975) *Tetrahedron Lett.* 1219–1222.
- Cavadore, J. C., Axelrud-Cavadore, C., Berta, P., Harricane, M. C. & Haiech, J. (1985) *Biochem. J.* **228**, 433–441.
- Chaponnier, C., Janmey, P. A. & Yin, H. L. (1986) *J. Cell Biol.* **103**, 1473–1481.
- Coue, M., Constans, J. & Olomucki, A. (1986) *Eur. J. Biochem.* **160**, 273–277.
- Doi, Y., Higashida, M. & Kido, S. (1987) *Eur. J. Biochem.* **164**, 89–94.
- Goldsmith-Clermont, P. S. V., Van Alstyn, E. L., Day, J. R., Emerson, D. L., Nel, A. E., Lazarchick, J. & Galbraith, R. M. (1986) *Biochemistry* **25**, 6467–6472.
- Hanin, V. (1990) Doctorat Thesis, University of Montpellier, France.
- Hanin, V., Naharisoa, H., Sarrade, V. & Calas, B. (1989) *Pept. Res.* **2**, 367–372.
- Harris, H. E. (1988) *FEBS Lett.* **233**, 359–362.
- Hirs, C. H. W. (1967) *Methods Enzymol.* **11**, 199–203.
- Janmey, P. A., Stossel, T. P. & Lind, S. E. (1986) *Biochem. Biophys. Res. Commun.* **136**, 72–79.
- Kabsch, N., Mannherz, H. G., Suck, D., Pai, E. F. & Holmes, K. C. (1990) *Nature (London)* **347**, 37–44.
- Kwiatkowski, D. J., Stossel, T. P., Orkin, S. H., Mole, J. E., Colten, H. R. & Yin, H. L. (1986) *Nature (London)* **323**, 455–458.
- Kwiatkowski, D. J., Mehl, R., Izumo, S., Nadal-Ginard, N. & Yin, H. L. (1988) *J. Biol. Chem.* **263**, 8239–8243.
- Kwiatkowski, D. J., Janmey, P. A. & Yin, H. L. (1989) *J. Cell Biol.* **108**, 1717–1726.
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
- Levine, B. A. & Moir, A. J. G. (1985) *J. Muscle Res. Cell Motil.* **6**, 76–77.
- Méjean, C., Roustan, C. & Benyamin, Y. (1987a) *J. Immunol. Methods* **99**, 129–135.
- Méjean, C., Boyer, M., Labbé, J. P., Marlier, L., Benyamin, Y. & Roustan, C. (1987b) *Biochem. J.* **244**, 571–577.
- Porte, F. & Harricane, M. C. (1986) *Eur. J. Biochem.* **154**, 87–93.
- Soua, Z., Porte, F., Harricane, M. C., Feinberg, J. & Capony, J. P. (1985) *Eur. J. Biochem.* **153**, 275–287.
- Spudich, J. A. & Watt, S. (1971) *J. Biol. Chem.* **246**, 4866–4871.
- Sutoh, K. & Yin, H. L. (1989) *Biochemistry* **28**, 5269–5275.
- Suzuki, R., Nishi, N., Tokura, S. & Morita, F. (1987) *J. Biol. Chem.* **262**, 11410–11412.
- Way, M. & Weeds, A. (1988) *J. Mol. Biol.* **203**, 1127–1133.
- Way, M., Gooch, J., Pope, B. & Weeds, A. G. (1989) *J. Cell Biol.* **109**, 593–605.
- Yin, H. L. (1988) *Bioessays* **7**, 176–179.
- Yonezawa, N., Nishida, E., Ohba, M., Seki, M., Kumagai, H. & Sakai, H. (1989) *Eur. J. Biochem.* **183**, 235–238.