A gelsolin-related protein from lobster muscle: cloning, sequence analysis and expression

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The tail muscle of the lobster *Homarus americanus* contains an actin-binding protein with an apparent molecular mass of 105 kDa determined by SDS/PAGE and gelsolin-like properties. We isolated this protein and peptide sequences were obtained after limited proteolysis with chymotrypsin. A tail-muscle-specific cDNA library was constructed in a λ expression vector and a full-length clone was obtained by screening with a polyclonal anti-(crustacean gelsolin) antibody. The cDNA insert of approx. 3.2 kb length was sequenced. The cDNA contained an open reading frame of 2.265 kb, and the deduced amino acid sequence of 754 residues (83469 Da) identified the protein as a cytoplasmic member of the gelsolin/villin protein family. Comparison of the lobster gelsolin amino acid sequence with other members of this protein family revealed the characteristic 6-fold repeated segmental structure as well as the three conserved sequence motifs typical of each segment [Way and Weeds (1988) J. Mol. Biol. **203**, 1127–1133]. Strong homologies were found with *Drosophila* gelsolin, human gelsolin, villin core, *Dictyostelium* severin and *Physarum* fragmin. In addition, the gelsolin-like protein from lobster muscle revealed motifs that were clearly similar to the actin-bundling region of human villin headpiece although it did not itself contain a distinct headpiece domain. The recombinant lobster gelsolin-like protein, expressed in *Escherichia coli* as a fusion protein, was purified from inclusion bodies and renatured as a functional protein. There were no significant differences in the biological activity tested between the recombinant and the native protein isolated from lobster muscle.

INTRODUCTION

Gelsolin is a Ca^{2+}-dependent multifunctional actin-binding protein which was first isolated from rabbit macrophages (Yin and Stossel, 1979). It belongs to a family of actin-binding proteins which also includes villin (Bretscher and Weber, 1979; Bazari et al., 1988; Arpin et al., 1988), fragmin from *Physarum* (Hasegawa et al., 1980; Hinssen, 1981; Ampe and Vandekerckhove, 1987), severin from *Dictyostelium* (André et al., 1988), an actin-modulating protein from annelid muscle (D'Haese and Hinssen, 1987), mbhI (Prendergast and Ziff, 1991) and adseverin (Sakurai et al., 1990; Nakamura et al., 1994).

Gelsolin shows a complex interaction pattern with actin: it severs actin filaments by interrupting actin–actin interactions within the filament, it binds two actin monomers thereby promoting actin nucleation, and it caps the fast polymerizing end of actin filaments. Activity is dependent on micromolar concentrations of Ca^{2+} (Yin and Stossel, 1979; Bryan and Kurth, 1984; Janmey et al., 1985; Bryan and Coluccio, 1985). Binding of G-actin is only partially reversible by EGTA as only one of the two bound actin monomers dissociates from the complex (Byran and Kurth, 1984; Janmey et al., 1985). Both reversibility of actin binding and inhibition of filament severing are mediated by phosphatidylinositides, especially phosphatidylinositol 4,5-bisphosphate (PIP_2) (Janmey and Stossel, 1987; Yin et al., 1988; Janmey et al., 1992; Yu et al., 1992).

Sequence analysis revealed a 6-fold segmental repeat (S1–S6) for human gelsolin with highly conserved motifs in each segment (Way and Weeds, 1988). The three actin-binding sites were localized more precisely by *in vitro* mutagenesis (Way et al., 1989, 1992a,b; Kwiatkowski et al., 1989). Severing activity is located in the N-terminal half of the molecule in segments S1–S3 (Way et al., 1989), and the actin-binding site in the C-terminal half [segments S4–S6 (Way et al., 1989)] contributes to the nucleating activity. Monomer binding sites are present in S1 and presumably in S4, and the F-actin-binding site is located in S2–S3 (Yin et al., 1988; Kwiatkowski et al., 1989; Way et al., 1989). By using synthetic peptides, putative PIP_2-binding sites were located in the border regions of S1 and S2 of gelsolin (Janmey et al., 1992; Yu et al., 1992). X-ray crystallography of gelsolin S1 and the S1–actin complex (McLaughlin et al., 1993) revealed that an α-helix in S1 binds to the actin monomer at the interface of the actin subdomains 1 and 3; two Ca^{2+} ions are also bound in this complex, one at a site formed by gelsolin alone, the other at a site formed by gelsolin and actin.

Villin is another member of the gelsolin protein family because its sequence also shows the 6-fold segmental repeat (Arpin et al., 1988; Bazari et al., 1988; Pope et al., 1994) but it has an additional 8 kDa 'headpiece' domain at the C-terminus. An additional actin-binding site in this headpiece enables cross-linking of actin filaments (Finidori et al., 1992; Friederich et al., 1992). At low Ca^{2+} concentrations ($\leqslant 10^{-7}$ M) villin acts as a bundling factor, and at higher Ca^{2+} concentrations ($\geqslant 10^{-6}$ M) villin function is similar to that of gelsolin (Glenney et al., 1980, 1981a,b; Bretscher and Weber, 1980). The distribution of villin seems to be restricted to cells that form microvilli (Bretscher et al., 1981; Robine et al., 1985).

In the present paper we report the isolation of a gelsolin-like protein from lobster tail muscle, the cloning and sequencing of a cDNA coding for this protein and its expression in *Escherichia*

Abbreviations used: DTE, 2,3-dihydroxybutane-1,4-dithiol; IPTG, isopropyl β-D-thiogalactoside; PIP_2, phosphatidylinositol 4,5-bisphosphate; poly(A)$^+$; polyadenylated; PVDF poly(vinylidene fluoride); TMAE-, tetramethylaminoethyl-.

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The nucleotide sequence data presented in this paper have been submitted to the GenBank and EMBL DataBank and DNA Database of Japan under the accession numbers L27814 (GenBank), Z29534 (EMBL) and D26582 (DNA Database of Japan).

coli. Although analysis of the derived amino acid sequence revealed strong homologies with vertebrate gelsolins, some motifs in the sequence were characteristic of villin rather than the gelsolins so far discovered, and therefore indicate a closer relationship to this type of protein. Biochemical data on the corresponding protein from the muscle of the crustacean *Astacus leptodactylus* (Bock et al., 1993, 1994) indicate that this actin-severing protein differed in some functional respects from the vertebrate gelsolins, as it formed complexes with three actin monomers in the presence of Ca^{2+}, and the binding of all three actins could be reversed by EGTA.

MATERIALS AND METHODS

Isolation of gelsolin-like protein from lobster tail muscle

Lobster gelsolin-like protein was isolated from the tail muscle by a method originally described for smooth muscle (Hinssen et al., 1984) with modifications introduced for preparation of gelsolin from the crayfish *A. leptodactylus* (Bock et al., 1994). All steps of the isolation and purification were monitored by SDS/PAGE (Laemmli, 1970). Gelsolin activity was assayed as described below.

Generation of anti-(crustacean gelsolin) antibody

A polyclonal antibody against crustacean gelsolin was raised using the corresponding protein from *A. leptodactylus* as antigen. The purified protein was subjected to preparative SDS/PAGE and the protein band transferred to nitrocellulose membrane by semi-dry blotting. After excision of the band, the membrane (containing approx. 2 mg of protein) was pulverized in liquid N_2 and resuspended in buffer (0.137 M NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.3). Rabbits were immunized with this suspension (0.5 mg of protein/injection; for the first two injections 0.2 mg/ml *N*-acetylmuramyl-L-alanyl-D-isoglutamine was added). The specificity of the polyclonal antiserum was verified by immunoblotting using total lobster tail muscle homogenate as a sample for SDS/PAGE.

Limited proteolysis with chymotrypsin and peptide sequencing

For limited proteolysis, lobster gelsolin was dialysed against buffer containing 10 mM imidazole, 0.2 mM EGTA, 0.5 mM 2,3-dihydroxybutane-1,4-dithiol (DTE) and 3 mM NaN_3 (pH 7.0). Immediately before incubation with protease, 1.2 mM $CaCl_2$ was added. Chymotrypsin was added (1:200, w/w, 25 °C) and the reaction was stopped at various times (30 s to 15 min) with 1 mM phenylmethanesulphonyl fluoride. The protein was subjected to SDS/PAGE and transferred electrophoretically to a poly(vinylidene difluoride) (PVDF) membrane. Protein bands of interest were excised and submitted to peptide sequencing by automated Edman degradation.

Immunoblotting

After SDS/PAGE the proteins were transferred to a nitrocellulose membrane by semidry blotting (Khyse-Anderson, 1984). The blot was blocked with 3 % gelatin and processed as described by Dissmann and Hinssen (1994), using an alkaline phosphatase-coupled secondary antibody for detection.

DNA and RNA techniques

Plasmid DNA was isolated using a plasmid kit (Quiagen). Total RNA was isolated from lobster tail muscle, and polyadenylated [poly(A)$^+$] RNA was purified using oligo(dT)–cellulose as described in Sambrook et al. (1989). The cDNA was synthesized and cloned into a λZAPII expression vector according to the manufacturer's instructions (Stratagene, La Jolla, CA, U.S.A.).

Standard procedures were used to screen the cDNA library with the anti-(crustacean gelsolin) antibody as described by Sambrook et al. (1989), using an alkaline phosphatase-conjugated secondary antibody in conjunction with chromogenic substrates. Unspecific cross-reactions of the antiserum with proteins of *E. coli* or λZAPII were eliminated by preincubation of the diluted antiserum on a plate lysate at 4 °C for 6 h. Positive plaques were cut out and rescreened two to three times. The pBluescript plasmid containing the cDNA insert was isolated from the phage by *in vivo* excision and was termed pLGS.

DNA sequences were determined by the dideoxynucleotide method of Sanger et al. (1977). Both strands were sequenced, using different primers.

Expression and purification of the recombinant lobster gelsolin-like protein

Lobster gelsolin was expressed in *E. coli* XL-1 Blue carrying the pLGS plasmid and induced with 1 mM isopropyl β-D-thiogalactoside (IPTG). The expressed protein was purified by the method of Nagai and Thøyersen (1987). The fusion protein was solubilized in buffer (0.1 M Tris, 0.1 M KCl, 8 M urea, 1 mM EGTA, 1 mM DTE, pH 7.6) and the extract dialysed against 2 × 100 vol. of 20 mM imidazole, pH 7.6, containing 1 mM EGTA, 150 mM KCl, 0.5 mM DTE and 0.5 mM $MgCl_2$. The protein renatured at least partially under these conditions, and the solution was centrifuged for 1 h at 100000 *g* to remove denatured protein. All steps of protein expression and purification were monitored by SDS/PAGE (Laemmli, 1970).

Measurement of lobster gelsolin-like protein activity

The biological activity of lobster gelsolin was assayed by Ostwald viscometry using skeletal-muscle actin (Hinssen et al., 1984). To assay fragmentation, 22 μM actin was polymerized in 10 mM imidazole, pH 7.4, containing 1 mM ATP, 100 mM KCl and 2 mM $MgCl_2$ for 30 min at 25 °C. Lobster gelsolin (0.5 μM) was added in the presence of either 0.5 mM $CaCl_2$ or 1 mM EGTA. Viscosity was measured within 5 min of the addition of gelsolin. To determine nucleating and capping activity, gelsolin was added to actin under the same conditions as above but before polymerization, and the specific viscosity was determined after 30 min of incubation at 25 °C. Gelsolin activity was expressed as percentage reduction in steady-state viscosity.

RESULTS AND DISCUSSION

Isolation of gelsolin-like protein from lobster muscle, sequencing of proteolytic peptides and specificity of the anti-(crustacean gelsolin) antibody

Gelsolin-like protein was isolated from lobster tail muscle as described in the Materials and methods section. Figure 1 shows the elution profile of the anion-exchange chromatography, the main purification step; the peak of activity was eluted at 300 mM KCl. The fractions containing activity were further purified by gel filtration on Ultrogel AcA34 followed by ion-exchange chromatography on TMAE-Lichrospher (not shown). An electrophoretically pure protein was obtained with a yield of 2–3 mg of protein/100 g of muscle.

Limited proteolysis of the purified protein with chymotrypsin initially generated two fragments of molecular mass 50 and

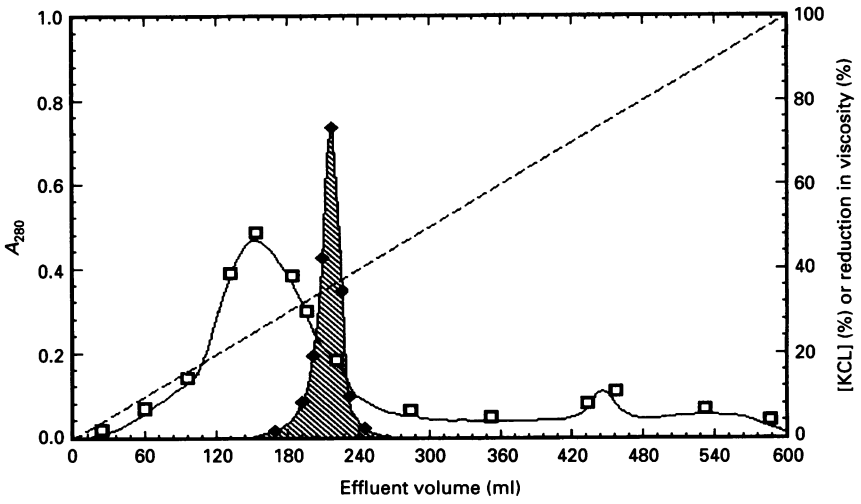

Figure 1 Isolation of gelsolin-like protein from lobster tail muscle: ion-exchange chromatography on DEAE-Sepharose

□, A_{280}; ----, linear salt gradient (150–500 mM KCl); ◆, fragmentation activity measured as percentage reduction in viscosity. Fractions with fragmentation activity were pooled (hatched area) and used for further purification by gel filtration on Ultrogel AcA34 and ion-exchange chromatography on tetramethylaminoethyl (TMAE)-Lichrospher.

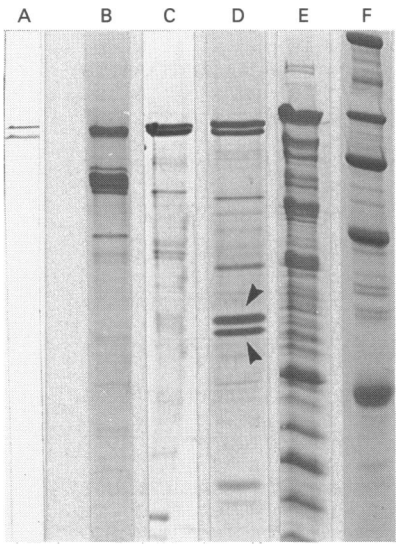

Figure 2 SDS/PAGE and immunoblot of total lobster muscle and selected samples from the protein preparation of native and recombinant lobster actin modulator

Lane A, immunoblot of a lobster tail muscle homogenate, incubated with the anti-(crustacean gelsolin) antibody; lane B, SDS/PAGE of the pooled fractions with actin-filament fragmentation activity after ion-exchange chromatography on DEAE-Sepharose; lane C, SDS/PAGE of the purified gelsolin-like protein from lobster muscle after ion-exchange chromatography on TMAE-Lichrospher; lane D, SDS/PAGE of the purified gelsolin-like protein from lobster tail muscle after limited proteolysis with chymotrypsin – the bands generated by the cleavage of the lobster protein are indicated by arrowheads; lane E, SDS/PAGE of total cell protein from *E. coli* after expression of the recombinant gelsolin-like protein; lane F, high-molecular-mass protein marker (210/116/97/67/43 kDa).

48 kDa (Figure 2, lane D). After reaction times of more than 20 min, the 50 kDa fragment disappeared and a distinct 14 kDa fragment together with several smaller fragments were generated (not shown). The proteolytic fragments were electroblotted from SDS/PAGE gel to PVDF membrane and subjected to automated Edman degradation. The 48 kDa fragment was N-terminally blocked but from the 50 and 14 kDa peptides two N-terminal sequences of 36 and 31 amino acids were obtained (Figure 3, underlined peptide sequences). The sequence data were used to verify the cDNA clone found by screening of the cDNA library. In addition, peptide sequences from the 50 and 14 kDa proteolytically generated fragments of the corresponding protein from the crayfish *A. leptodactylus* were obtained (results not shown). The sequence for the 14 kDa fragment (31 amino acids were obtained from the crayfish protein) was the same as for lobster and only two of 25 amino acids were different for the 50 kDa fragment. We therefore assume that the two proteins are almost identical.

The generated antibody appeared to be crustacean-specific and did not cross-react with vertebrate gelsolins (results not shown). In immunoblots with samples of total lobster muscles, the antibody recognized only two bands of molecular mass 105 and 100 kDa (Figure 2, lane A). The purified protein also showed two components on SDS/PAGE (Figure 2, lane D) of molecular mass 100 and 105 kDa. Immunoblots of these samples showed that the antibody in fact reacted with both polypeptides (not shown). The existence of two isoforms of this protein in lobster is suggested on the basis of these data. Although an extracellular and a cytoplasmic gelsolin variant have been identified in *Drosophila* cDNA (Heintzelman et al., 1993; Stella et al., 1994), it seems unlikely that the two lobster gelsolin isoforms represent similar variants; as will be discussed below, we have cloned the larger isoform which does not have the N-terminal extension typical of the extracellular form. In addition, we have found no cross-reaction of the antibody with lobster haemolymph fluid in immunoblots (results not shown).

Bock et al. (1994) have investigated the interaction mechanisms of the purified gelsolin-like protein from *Astacus* with actin, and the data revealed several unusual properties for this actin-modulating protein. (1) In the presence of Ca^{2+}, it formed complexes with three actin monomers, indicating the possibility of a third actin-binding site, whereas vertebrate gelsolin binds only two actins. (2) The interaction of the crayfish protein with

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1                                   M  V  P  A  F  E  G  A  G  A  V  E  G  L  T  I  W
1       CTTGTCAGCTGCACACCATGGTTCCTGCCTTCGAGGGAGCTGGTGCCGTGGAGGGCCTGACCATCTGG
18       R  I  E  N  F  E  V  V  P  Y  P  K  E  K  Y  G  Q  F  Y  Q  G  D  S
69      AGGATCGAGAACTTCGAGGTGGTTCCATACCCTAAGGAAAAGTACGGCCAGTTCTACCAGGGGGATTCC
41       Y  I  V  L  Y  T  R  D  V  N  G  N  L  S  W  D  L  H  F  W  L  G  S
138     TACATCGTCCTCTATACAAGAGATGTGAATGGAAACTTAAGCTGGGACCTGCACTTCTGGTTAGGTTCG
64       E  T  S  Q  D  E  A  G  T  A  A  1  K  T  V  E  L  D  D  Q  L  G  G
207     GAAACATCTCAGGATGAAGCCGGTACAGCAGCGATCAAGACGGTGGAGTTGGACGACCAGCTGGGCGGT
87       V  P  V  Q  H  R  E  V  E  G  H  E  T  S  L  F  L  S  R  F  K  K  G
276     GTTCCTGTCCAGCACAGAGAGGTGGAGGGCCACGAGACCTCCCTCTTCCTCTCCAGGTTCAAGAAGGGG
110      V  R  Y  L  K  G  G  V  A  S  G  F  H  H  V  D  P  D  A  P  Y  P  A
345     GTGAGGTACCTGAAGGGGGGTGTGGCCTCTGGTTTCCATCACGTGGACCCAGATGCACCTTACCCTGCT
133      R  L  F  H  V  K  G  R  R  N  I  R  I  R  Q  V  E  V  G  V  G  S  M
414     CGCCTCTTCCACGTCAAGGGCCGCAGGAACATCCGTATCAGACAGGTTGAAGTTGGTGTAGGATCCATG
156      N  K  G  D  C  F  I  L  D  C  G  S  Q  V  Y  A  Y  M  G  P  S  S  R
483     AACAAGGGTGACTGCTTCATCCTGGACTGTGGCTCACAAGTCTATGCATACATGGGGCCAAGCAGCAGG
179      K  M  D  R  L  K  A  I  Q  A  A  N  P  V  R  A  D  D  H  A  G  K  A
552     AAGATGGACCGTCTCAAGGCCATCCAGGCGGCAAATCCTGTGAGGGCCGATGATCATGCTGGAAAGGCT
202      K  V  I  V  I  D  E  T  A  S  G  S  E  A  G  E  S  S  P  G  L  G  G
621     AAAGTCATTGTTATTGATGAAACTGCATCAGGAAGTGAGGCGGGGGAGTCCTCCCCTGGCCTAGGTGGT
225      G  S  P  D  D  V  A  D  E  D  T  G  V  D  D  S  A  F  E  R  S  E  V
690     GGTTCCCCTGATGACGTCGCTGATGAAGATACTGGTGTTGATGACTCTGCCTTTGAGCGGTCTGAAGTG
248      N  V  V  T  L  H  H  I  F  E  D  G  D  G  V  I  Q  T  N  M  I  G  E
759     AATGTGGTAACACTGCATCACATCTTTGAGGATGGTGATGGTGTTATTCAGACAAACATGATTGGTGAG
271      K  P  L  L  Q  S  M  L  D  S  G  D  C  F  L  L  D  T  G  V  G  V  Y
828     AAGCCTCTGCTGCAGTCCATGCTCGACTCTGGTGACTGTTTCCTTCTTGACACTGGTGTTGGAGTGTAT
294      V  W  I  G  S  G  S  S  K  K  E  K  V  K  S  M  E  L  A  A  G  Y  M
897     GTGTGGATTGGCAGCGGTTCCAGCAAGAAGGAGAAGGTCAAGAGTATGGAGTTGGCTGCTGGCTATATG
317      E  K  K  G  Y  P  T  Y  T  N  V  Q  R  V  V  E  K  A  E  P  A  V  F
966     GAGAAGAAAGGATATCCCACATACACGAACGTACAGCGTGTGGTTGAGAAGGCTGAGCCAGCGGTGTTC
340      K  A  Y  F  K  T  W  R  E  P  Q  E  Q  I  G  L  G  R  V  F  T  Q  R
1035    AAGGCCTACTTCAAGACATGGAGGGAACCTCAAGAACAAATTGGACTTGGTCGCGTCTTTACCCAGCGA
363      Q  M  S  A  V  S  A  T  E  T  D  F  D  V  S  S  L  H  A  E  K  R  R
1104    CAGATGTCTGCAGTGTCTGCTACAGAGACAGACTTCGACGTTAGCTCCTTGCATGCAGAGAAGCGCCGT
386      L  L  Q  K  N  A  G  P  A  F  A  L  C  P  I  M  V  L  A  R  R  N  L
1173    TTGTTGCAGAAGAATGCTGGTCCGGCCTTTGCTTTATGCCCGATAATGGTTCTGGCAAGACGGAACTTG
409      G  P  L  R  T  L  K  L  E  P  V  D  E  S  T  H  G  F  F  F  G  G  D
1242    GGCCCGTTGAGAACTTTGAAGCTTGAACCAGTGGATGAAAGCACTCATGGCTTCTTCTTCGGTGGTGAT
432      S  Y  V  L  K  Y  I  Y  E  V  N  G  N  E  R  Y  I  L  Y  F  W  Q  G
1311    TCATACGTCCTGAAGTACATCTATGAGGTTAACGGCAACGAACGCTACATCCTCTACTTTTGGCAGGGT
455      C  A  S  S  Q  D  E  K  A  S  S  A  I  H  T  V  R  L  D  N  E  L  C
1380    TGTGCCAGTAGCCAGGACGAGAAGGCCTCTTCAGCCATCCACACTGTCCGCCTGGATAATGAACTGTGT
478      G  K  A  V  Q  V  R  V  V  Q  G  Y  E  P  A  H  F  L  R  I  F  K  G
1449    GGCAAGGCTGTTCAAGTTCGTGTTGTTCAGGGGTATGAGCCGGCACACTTCCTCAGAATTTTCAAGGGT
501      R  M  V  I  F  L  G  G  K  A  S  G  F  K  N  V  H  D  H  D  T  Y  D
1518    CGTATGGTCATTTTCCTCGGCGGTAAGGCTTCTGGCTTCAAGAATGTGCATGATCATGACACTTATGAT
524      V  D  G  T  R  L  F  R  V  R  G  T  C  D  F  D  T  R  A  I  Q  Q  T
1587    GTTGACGGCACAAGGCTATTCCGGGTGCGCGGGACATGTGACTTCGATACCCGTGCCATCCAGCAGACT
547      E  V  A  G  S  L  N  S  D  D  V  F  V  L  E  T  P  G  K  T  Y  L  W
1656    GAGGTAGCCGGCTCTCTCAACTCCGACGATGTCTTTGTCTTGGAAACCCCAGGGAAGACTTACCTCTGG
570      I  G  K  G  A  S  E  E  E  K  A  M  G  E  K  V  V  E  L  V  S  P  G
1725    ATTGGCAAGGGAGCAAGTGAAGAGGAGAAGGCTATGGGTGAAAAGGTTGTCGAGTTGGTGTCTCCAGGT
593      R  D  M  V  T  V  A  E  G  E  E  D  D  D  F  W  G  G  L  G  G  K  G
1794    CGTGACATGGTGACTGTTGCTGAAGGAGAGGAAGATGATGATTTCTGGGGTGGTCTTGGAGGCAAGGGT
616      D  Y  Q  T  A  R  D  L  D  R  P  L  L  Y  P  R  L  F  H  C  T  I  S
1863    GACTACCAGACTGCTCGTGATCTGGACAGACCTCTGCTGTACCCCAGACTCTTCCACTGCACCATCTCC
639      P  A  G  C  L  R  V  N  E  M  S  D  F  A  Q  E  D  L  N  E  D  D  V
1932    CCTGCTGGCTGCCTCAGGGTTAATGAGATGTCTGACTTTGCCCAGGAGGATCTGAATGAAGATGATGTT
662      M  V  L  D  S  G  D  E  V  Y  V  W  V  G  Q  G  S  D  D  Q  E  K  E
2001    ATGGTGCTGGACTCTGGTGATGAAGTATATGTTTGGGTGGGACAAGGTTCAGATGACCAGGAGAAAGAG
685      K  A  F  T  M  A  E  N  Y  I  K  T  D  P  T  E  R  T  L  D  A  T  V
2070    AAAGCCTTCACAATGGCAGAGAACTACATTAAGACCGACCCAACTGAGCGCACCTTAGACGCTACCGTG
708      I  L  R  I  N  Q  G  E  E  P  A  A  F  T  S  I  F  P  A  W  N  P  D
2139    ATCCTGCGCATCAACCAGGGAGAGGAGCCGGCAGCATTCACGTCCATCTTCCCTGCCTGGAACCCAGAC
731      M  W  Q  K  G  L  V  S  Y  D  D  M  K  A  Q  V  P  E  T  N  A  A  V
2208    ATGTGGCAGAAGGGGCTGGTTAGCTACGACGACATGAAGGCACAGGTGCCGGAGACGAATGCTGCCGTT
754      E  *
2277    GAGTGAGAACCTACAATATGACAAGGACAGAGGGCGAAGATTCTGCACAAAAATAGAAGTGAAGGAGGA
2346    GAAAATAACCAAATTTTATAATCAATGAGTTTAAGGGGAGGGCGAGGGGTATTATATGTTTTCCTGATG
2415    ATGCTGTGTCTAAGTGCTGTATATTGACTTTATATTCAAGTTTAGCCTCTCAATACAGTACTGTATAAA
2484    ACAGGATTTAAGTACGTAGTAAGTTTACTTTGTGATATTGATTTCTATCTTAGTGGGGTTTTTTTCTCT
2553    CTCTTAATTGGGACTTCAGGCAATGGTATAACACTTATGTTTGAGATTTTGTTGATCTTAAAGAAATTT
2622    TTATGAAACAAAAATGGTTAAATTTCAGTTCTTGGATAACTACAGTGATCCTTGTATTACATAGTGTAA
2691    TGGATATACAGATTAGATGTGTTGGTCTGATGGTGCTTGATGCTATTGCCGGTCATGGTCAGTGGGAC
2760    CTCCTGTAGATTTTCAACTTTTAGTGCCTTAAGTGTCCAGTTTTCAAAATACACAGCACATTTTATAAT
2829    ATTATTGTATGCCTTCGTAACTCTTGCCAGCAAAGTATTATAGTAGATCTCGTTAAAATTTAATGTAGA
2898    AGTGTTACTGCCTATGGAATGATGCATTTTTAGAGCATTTATGTGCACAGAATACCTTGTAGACAGAGG
2967    AAACAGTTTGTTAAAGAAATATTTGATCATGATAAACTGATTTCTAAGTATTTTAATCATAATATAAAA
3036    ACTTTGAGTAGTAAGTAAGTACATAATAATATTATATACTTGGCTTCATGGTCAGAAATGGCAGATACA
3105    TAATATTACATTTCAATATTTACTTTCAGCTTGTTTGGTTGTCTATCGGCTCAGTCTCTTAATATCTTG
3174    GCCTTTGAAAAATCTCCTTCCATTATCCCAGGCTTAAGTGTGTTCAAG
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actin could be completely reversed by EGTA, whereas in vertebrate gelsolin only one of the two actins bound can be removed under these conditions. (3) The actin-modulating protein from *A. leptodactylus* was able to sever actin filaments even after preforming stoichiometric complexes with actin, which is impossible for vertebrate gelsolin. It was therefore of interest to investigate whether any of these specific aspects would be reflected in the structure of crustacean gelsolin-like proteins.

Construction and screening of the muscle-specific cDNA library from lobster

From 5 μg of poly(A)$^+$ RNA a yield of 770 ng of cDNA was obtained and the length of the cDNA strands reached from $\approx$ 100 bp to $\approx$ 4.0 kbp with a maximum of synthesis at 1.5–2 kb. Accordingly 140 ng of cDNA was ligated to 1.4 μg of λZAPII vector arms. After construction of the primary library by *in vitro* packaging of the recombinant λ phages, titering revealed a complexity of about 1.1×10^6; the percentage of non-recombinant molecules was 1.5%, determined by the 5-bromo-4-chloroindol-3-yl β-D-galactopyranoside ('X-Gal') test. Screening of the library with the polyclonal antibody to crustacean gelsolin produced a cDNA clone with an insert of approx. 3.2 kb in length.

Sequence analysis

The recombinant plasmid, termed pLGS (for *l*obster *g*el*s*olin), is a derivative of the Bluescript SK plasmid and is 6175 bp in length. The cDNA insert was fused to the *lacZ'* region of bacterial β-galactosidase gene at its 5' end, so, when expressed, a fusion protein with an N-terminal extension of 47 amino acids is synthesized. The first 37 amino acids are the fusion part of β-galactosidase; ten amino acids are contributed by both the *Eco*RI linker (13 bp) and the part of the 5'-non-coding region of the cDNA (17 bp).

The cDNA was sequenced throughout its whole length in both directions, starting with the T3 primer for the coding strand and the T7 primer for the complementary strand. The cDNA has a length of 3221 bp and includes 17 bp from the 5'-non-coding region, an open reading frame of 2265 bp and 939 bp from the 3'-non-coding region (Figure 3).

The open reading frame starts at base position 18–20 with the ATG start codon and terminates at position 2282–2284 with a TGA stop codon, encoding a protein of 754 amino acids in length. In the 3'-non-coding region, one poly(A)$^+$ site is found at position 2349–2353 (AATAA, underlined in Figure 3). This signal is different from the poly(A)$^+$ signals found mainly in eukaryotic transcripts (AATAAA) but has been described previously (Arpin et al., 1988).

DNA sequencing also revealed that the poly(A)$^+$ sequence was missing at the 3' end of the cDNA. This was somewhat puzzling, as processing of cDNA during the synthesis should lead to different cDNA ends (*Eco*RI–*Xho*I) with the *Xho*I restriction site downstream of the poly(A)$^+$ sequence. One possible explanation is that the last six nucleotides represent a mutated *Xho*I site [there are only two differences in the cDNA sequence compared with a *Xho*I site: **TTCAAG** is the sequence found in the cDNA and the normal *Xho*I site is **CTCGAG** (differences in bold)]. It cannot be ruled out that this site was initially an additional *Xho*I site which was not protected by 5-methylcytosine (this modified nucleotide is used in cDNA synthesis, particularly in first-strand synthesis to protect internal *Xho*I sites) so that *Xho*I cleaved the cDNA at this position.

The deduced amino acid sequence of the open reading frame was verified by the amino acid sequences obtained from the 50 and 14 kDa peptides. There were no differences except for an X in the amino acid sequence of the 14 kDa fragment at position 23 which corresponds to a C in the open reading frame. Cysteine was possibly not identified by peptide sequencing because of its reactive SH group, which might have been destroyed during the analysis.

The protein encoded by the open reading frame is 754 amino acid residues long and has a calculated molecular mass of 83469 Da. Altogether the protein has 197 charged amino acids, 117 negative and 80 positive. The excess of 37 negative charges explains the low isoelectric point (about 5.0–5.1; determined by two-dimensional electrophoresis; results not shown) and may cause unusually low binding of SDS, explaining the abnormal mobility on SDS/PAGE (apparent molecular mass determined by SDS/PAGE, 105 kDa). The overall amount of charged amino acids is somewhat higher than in vertebrate gelsolin (pI 6.1): 26.1 compared with 24% of charged amino acids in total, 10.6 compared with 11% positively charged and 15.5 compared with 13% negatively charged amino acids.

Sequence analysis of the isolated full-length cDNA clone from lobster clearly identified the encoded protein as a member of the gelsolin family. As we have not found any evidence for a plasma extension, it is considered to be a cytoplasmic gelsolin-like protein. This is at variance to the results of Heintzelman et al. (1993), who identified a cDNA encoding an extracellular form of gelsolin in *Drosophila melanogaster*. However, Stella et al. (1994) also identified a cytoplasmic gelsolin variant in *Drosophila*. It may be argued that the known sequence upstream from the putative translational start site (ATG from position 18–20 in cDNA) is too short to decide whether the lobster protein is a plasma or a cytoplasmic variant. Although the possibility of a plasma gelsolin cannot be completely excluded, the hexanucleotide sequence found immediately adjacent to the presumed translational start site (CACACC) corresponds to those reported by Kozak (1984), who investigated the sequences upstream from the translational start site. A similar sequence motif was also found outside the coding sequence of the cDNA of the secretory gelsolin from *D. melanogaster* (Heintzelman et al., 1993).

Homology analysis

In Table 1 the amino acid sequence of the lobster protein was compared with those of other members of the gelsolin protein family. The highest values of similarity and identity were found by comparing the lobster protein to *Drosophila* gelsolin but a considerable degree of identity was also found with human and pig gelsolin. The lowest values are obtained for protovillin (Hofmann et al., 1993). As fragmin and severin are only half the size of the lobster protein, the comparison was carried out in these cases with the N-terminal half of lobster gelsolin (amino acids 1–365). Comparison of fragmin and severin with the C-terminal half of the lobster actin modulator produced signifi-

Figure 3 Nucleotide sequence of the cDNA insert coding for the gelsolin-like protein from lobster tail muscle

The deduced amino acid sequence is shown above the nucleotide sequence using the one-letter code. Numbers at the beginning of each lane refer to nucleotide and amino acid positions. The sequence determining the translational start site [CACACC (Kozak, 1984)] and a putative poly(A)$^+$ site (AATAA) are underlined. Underlined amino acid sequences (amino acids 360–395 and 514–544) were obtained by peptide sequencing. The codon terminating the open reading frame is shown as an asterisk. The missing poly(A)$^+$ sequence is discussed in the text.

Table 1 Homology of the lobster gelsolin-like protein to other members of the gelsolin family

Values for homology and identity respectively were obtained by comparing the amino acid sequences of the different proteins to the lobster actin modulating protein by the alignment procedure described in Figure 4.

	Homology (%)	Identity (%)
Drosophila gelsolin	57	43
Human gelsolin	53	41
Pig gelsolin	52	40
Human villin	52	38
Protovillin	47	32
Fragmin	49	38
Severin	48	38

cantly lower values of 28 and 29% identity and 33 and 36% similarity respectively (results not shown).

It should be noted that the distribution of homologous regions is not homogeneous throughout the sequence. The highest degree of both similarity and identity in all known gelsolin-like proteins is found within the first 200 amino acids which represent the S1 segment and about half of the S2 segment. Another relatively conserved region is found between amino acids 450 and 700 in these proteins (results not shown), indicating the importance of certain conserved parts of the molecule for protein function.

All known proteins of the gelsolin family are characterized by segmentally repeated structures (6-fold for gelsolin and villin and 3-fold for fragmin, severin and mbhI) with a set arrangement of conserved motifs [termed B, A and C (Way and Weeds, 1988)]. Alignment of the amino acid sequence of the lobster protein with the conserved regions of other members of this protein family (Figure 4) reveals the 6-fold segmental repeat, and the motifs A, B and C are readily recognized. The conserved regions are in good accord with those of the other proteins. In addition, we compared the significant sequence elements of human gelsolin S1 with the corresponding amino acid sequences of lobster gelsolin-like protein (Figures 5a–5e).

Although the amino acids that probably form the hydrophobic core of the molecule are well conserved (Figure 5a), just as the putative actin-binding helix is (residues 72–84, Figure 5b), the additional introduction of charges in the putative actin-binding α-helix may affect the interaction with actin. If these residues form an ionic interaction this may result in weaker actin binding of the lobster actin modulator relative to gelsolin.

The F-actin-binding site in gelsolin is located within segments S2 and S3 (Bryan, 1988; Yin et al., 1988; Kwiatkowski et al., 1989; Way et al., 1989). Sun et al. (1994) have pinpointed amino acids 161–172 of human plasma gelsolin as this binding site. In the corresponding sequence of the lobster protein (Figure 5c) we

	B	A	C	
Lobster gelsolin	(35) F YQG D S YIV	(57) L HF W L G SETSQD E AGT A	(95) EG H ET SL FL S RF	
Drosophila gelsolin	(85) F YTG D S FIV	(109) V HF W L G LETSTD E AGA A	(147) QD H ES QL FL S YF	
Human gelsolin	(89) F FTG D A YVI	(112) L HY W L G NECSQD E SGA A	(150) QG F ES AT FL G YF	Segment 1
Villin	(40) F FDG D C YII	(62) I HY W I G QDSSLE Q GAA A	(100) QG N ES EA FR G YF	
Fragmin	(66) F YTG D S YIV	(91) V HF W L G AFTTQD E AGT A	(129) QG Y ES ER FL S LF	
Severin	(66) F YDG D S YII	(88) I HF F L G TFTTQD E AGT A	(126) QG N ES DL FM S YF	
Lobster gelsolin	(155) M NKG D C FIL	(169) V YA Y M G PSSRKM D RLK A	(208) ET A SG SE AG E SS	
Drosophila gelsolin	(206) M NTG D C FIL	(221) I YV Y V G SOAKRV E KLK A	(266) ES T AD ED SA F ER	
Villin	(210) F NNG D C FIL	(224) I HQ W C G SNSNRY E RLK A	(263) EG T EP EA ML Q VL	
Human gelsolin	(160) F NRG D V FLL	(174) I IQ W N G PESTRM E RLR G	(216) EL A SP -K LM E VM	Segment 2
Fragmin	(177) L NSG D V FVL	(191) V IQ W N G AKAGLL E KVK A	(230) AD N DT -E FF T LL	
Severin	(184) L NSG D C FLL	(198) I YQ F N G SKSSPQ E KNK A	(238) DS D IP AE FW K LL	
Lobster gelsolin	(278) L DSG D C FLL	(292) V YV W I G SGSSKK E KVK S	(332) EK A EP A VF -KA YF	
Drosophila gelsolin	(329) L DTR E C FIL	(343) I FV W V G KGATQK E KTD A	(383) EG S ES A PF -KQ YF	
Villin	(326) L KSE D C FIL	(342) I FV W K G KQANTE E RKA A	(382) EG G ET P LF -KQ FF	
Human gelsolin	(280) L SHE D C YIL	(295) I YV W K G KKANEQ E KKG A	(335) DG A ES A VF -QQ LF	Segment 3
Fragmin	(293) L DSN D V FVL	(308) V FA W V G KHASVG E KKK A	(347) EG G EN E VE FED FF	
Severin	(295) L KSE D V FIL	(309) I YT W I G SKSSPN E KKT A	(349) EN G TN Q SF FET LL	
Lobster gelsolin	(427) F FGG D S YVL	(449) L YF W Q G CASSQD E KAS S	(487) QG Y EP AH FL R IF	
Drosophila gelsolin	(488) T YNY E A NNG	(502) T YV W H G VKASAA A RKR A	(536) QG H EP RH FY K IF	Segment 4
Human gelsolin	(468) F YGG D S YII	(490) I YN W Q G AQSTQD E VAA S	(528) QG K EP AH LM S LF	
Villin	(421) F YGG D C YLL	(443) L YV W Q G SQASQD E ITA S	(481) MG K EP PH LM S IF	
Lobster gelsolin	(552) L NSD D V FVL	(566) T YL W I G KGASEE E KAM G	(600) EG E ED DD FW G GL	
Drosophila gelsolin	(587) L ASS D A LFV	(603) I YI W N G LGASAF E KQA A	(637) EG A EP DE FW E EL	Segment 5
Human gelsolin	(588) L NSN D A FVL	(602) A YL W V G TGASEA E KTG A	(634) EG S EP DG FW E AL	
Villin	(540) L NSN D V FVL	(554) C YL W C G KGCSGD E REM A	(587) EG Q EP AN FW M AL	
Lobster gelsolin	(656) L NED D V MVL	(670) V YV W V G QGSDDQ E KEK A	(713) QG E EP AA FT SI FP	
Drosophila gelsolin	(694) L DSD D I MLL	(708) I YL W V G YGVSEE E NGK L	(751) QG K EP RV FK RM FP	Segment 6
Human gelsolin	(693) L ATD D V MLL	(707) V FV W V G KDSQEE E KTE A	(749) QG F EP PS FV GW FL	
Villin	(644) L EED D V FLL	(658) V FF W I G KHANEE E KKA A	(700) QG H EP PT FT GW FL	

Figure 4 Alignment of conserved motifs in all segments of gelsolin-related proteins according to the nomenclature of Way and Weeds (1988)

Sequences of lobster gelsolin (this paper), *Drosphila* gelsolin (Heintzelman et al., 1993), human gelsolin (Kwiatkowski et al., 1986), human villin (Arpin et al., 1988), fragmin (Ampe and Vandekerckhove, 1987) and severin (André et al., 1988) are aligned. Numbers in parentheses indicate the position of the first amino acid in the respective motif. The conserved amino acids in the motifs are in bold.

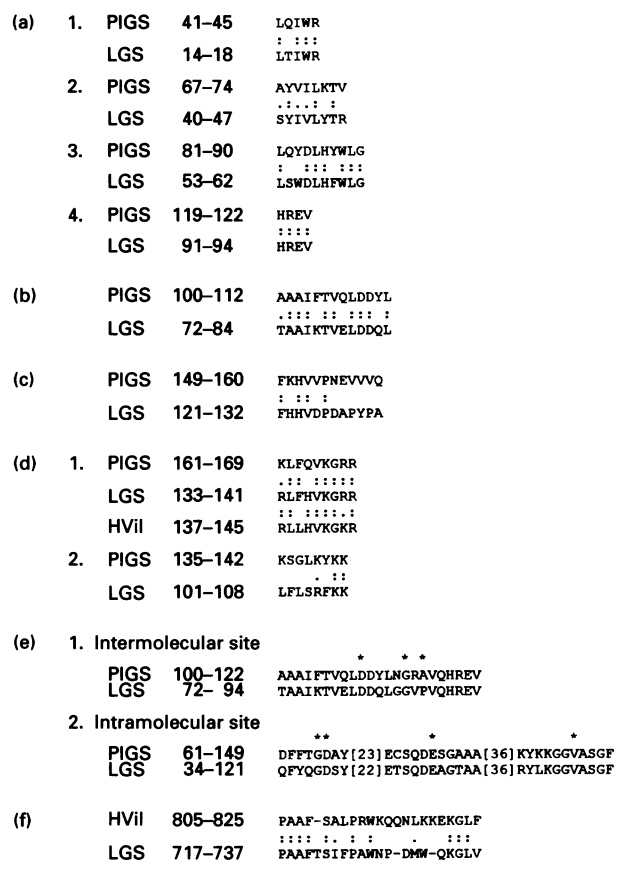

Figure 5 Alignment of important amino acid sequences in gelsolin S1 (McLaughlin et al., 1993) and human villin headpiece (Arpin et al., 1988) with corresponding sequence motifs on the gelsolin-like protein from lobster tail muscle

Identical amino acids are indicated by ':', amino acids changed by conservative replacement are indicated by '·'. PIGS, human plasma gelsolin (McLaughlin et al., 1993); LGS, lobster gelsolin-like protein; HVil, human villin. Conservative replacements are defined in five groups: 1, I, L, V, M; 2, A, S, T; 3, K, R; 4, D, E; 5, N, Q. Alignment parameters used were: matched score, 2; mismatch penalty, 4; gap penalty, 1. Alignments were performed with the ALIGN PLUS program (Scientific and Educational Software Inc.). (**a**) Amino acids forming the four-stranded hydrophobic core of gelsolin S1. (**b**) Amino acids forming the actin-binding α-helix of gelsolin S1. (**c**) Amino acids of gelsolin involved in F-actin binding (Sun et al., 1994). (**d**) PIP_2-binding sites of gelsolin and villin (Janmey et al., 1992; Yu et al., 1992) in the border regions of segments S1 and S2. (**e**) Ca^{2+}-co-ordinating sites in gelsolin S1 (McLaughlin et al., 1993). Amino acids involved in the co-ordination of the bound Ca^{2+} are marked by an asterisk. Numbers in square brackets indicate the distance between sequences in amino acids. (**f**) Amino acids involved in the bundling activity of villin headpiece (Friederich et al., 1992) compared with a similar sequence found in the lobster gelsolin-like protein.

also found a high degree of similarity to human plasma gelsolin with nine of 12 identical amino acid residues and one conservative replacement. This sequence motif contains in addition one of the putative PIP_2-binding sites (see Figure 5d), so lateral binding of the lobster actin modulator to F-actin may be regulated effectively by PIP_2.

Interaction of gelsolin and villin with F-actin is affected by PIP_2, which inhibits binding to F-actin (Janmey and Stossel, 1987; Yin et al., 1988; Janmey et al., 1992; Yu et al., 1992). Using synthetic peptides, Janmey et al. (1992) and Yu et al. (1992) found two PIP_2-binding regions in human gelsolin (amino acids 135–142 and 161–169) but only one in human villin (amino acids 137–145). In lobster actin modulator, two putative PIP_2-binding sites were identified (Figure 5d). One of them (amino acids 133–141) is highly similar to that of both human gelsolin and villin.

The other presumptive PIP_2-binding site in the lobster actin modulator was more difficult to identify; the first half of this motif does not fulfil the requirements for the consensus sequence (Yu et al., 1992), as the first amino acid of this motif should be a positively charged amino acid residue. In lobster gelsolin-like protein there is a leucine in this position. It may be possible that this change in the sequence causes a relative insensitivity to regulation via PIP_2 at this binding site. However, as the interaction between lobster gelsolin and actin is fully EGTA-reversible, the physiological relevance of PIP_2-mediated regulation may be less than that of vertebrate gelsolins.

The activity of all known gelsolin-related proteins is dependent on the presence of Ca^{2+}. The structural data for the S1 segment of human gelsolin reveal the existence of two Ca^{2+}-binding sites (McLaughlin et al., 1993), one intermolecular site formed by gelsolin and actin and an intramolecular site formed by gelsolin S1 alone. A possible Ca^{2+}-binding site in lobster actin modulator may be formed by the amino acids Asp^{39} and Glu^{68} (side chains), Gly^{38} and Val^{116} (peptide bonds) and one molecule of water. The intermolecular Ca^{2+}-binding site may consist of amino acids Asp^{81} (side chain), Gly^{86} and Pro^{88} (peptide bonds) and the side chain of Glu^{167} of actin (Figure 5e). On the basis of these data, which show a high degree of conformity compared with the gelsolin structure, a close relationship between the lobster actin modulator and the gelsolin protein family is strongly suggested.

At the C-terminal region of the lobster gelsolin-like protein we found a sequence motif very similar to the part of the villin headpiece directly involved in actin-bundling activity [Figure 5f (Friederich et al., 1992)]. In the sequence of human villin, the very basic tetrapeptide KKEK is essential for actin bundling, which was completely abolished by its removal. Although the KKEK motif is crucial for filament bundling, it has been shown that all amino acids between Ser^{809} and Lys^{823} are required for complete functionality (Friederich et al., 1992). We have found elements of this sequence in lobster actin modulator (Figure 5f) at two positions, although not in 'headpiece'-like C-terminal domain. A KKEK tetrapeptide is located in the third segment at amino acid position 302–305. Its surrounding region is very different from that of the human villin headpiece sequence but it cannot be excluded that this sequence, if exposed on the surface of the molecule, may function as part of an actin-binding site. In addition, elements of the C-terminal sequence of human villin (Arpin et al., 1988) appear in lobster gelsolin at its C-terminus with ten of 22 amino acids being identical (Figure 5f).

The KKEK motif of human villin is modified in the C-terminus of lobster actin modulator. Nevertheless the critical lysine residue (Lys^{823} for human villin and Lys^{734} for lobster actin modulator) for actin binding (Friederich et al., 1992) is the correct distance away from the PAAF tetrapeptide. Alteration of KKEK may cause loss of actin filament cross-linking. Despite its homology with the villin C-terminus, the lobster actin-modulating protein has no headpiece: as seen in Figure 4, part of the 21-amino acid sequence in lobster gelsolin belongs to the conserved motif C in segment 6 and is therefore an integral part of the 'core' structure. However, in none of the other gelsolins or in villin itself does there exist such a high degree of homology with the villin headpiece motif as in the lobster actin modulator.

From sequence analysis alone it is not possible to locate a third actin-monomer-binding site. One possible candidate for this site is the KKEK tetrapeptide [as it is in the human villin headpiece (Friederich et al., 1992)] in the middle of the lobster molecule. This is not impossible, as Pope et al. (1994) demonstrated that, in villin, the actin-binding sites in segments 2 and 3 and in the

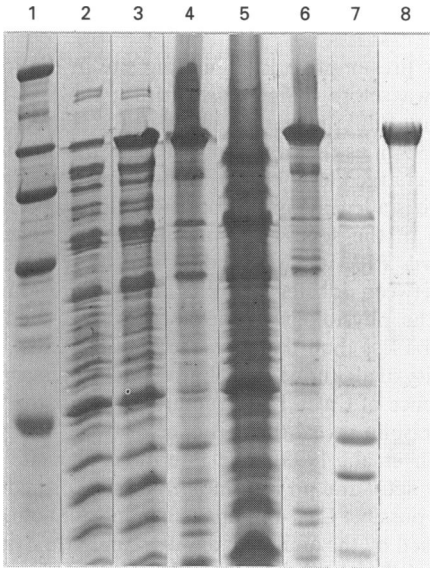

Figure 6 Expression and purification of lobster gelsolin-like protein in *E. coli* assessed by SDS/PAGE

Lane 1, high-molecular-mass protein marker (210/116/94/67/43 kDa); lane 2, total cell protein before induction; lane 3, total cell protein 3 h after induction; lane 4, insoluble protein after lysis of bacteria; lane 5, soluble protein after lysis of bacteria; lane 6, insoluble protein in 0.5% Triton X-100/1 mM EDTA; lane 7, soluble protein in 0.5% Triton X-100/1 mM EDTA; lane 8, purified recombinant gelsolin-like protein, soluble in 8 M urea.

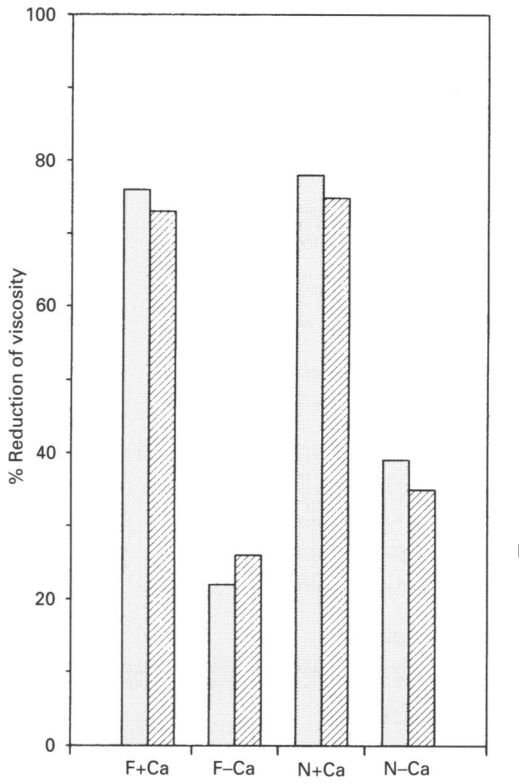

Figure 7 Biological activity of recombinant lobster gelsolin-like protein compared with native protein isolated from lobster muscle

Percentage reduction in viscosity is used as a measure of filament severing (fragmentation, F) and nucleation (N) in the presence of either 0.5 mM Ca^{2+} (F + Ca^{2+}; N + Ca) or 1 mM EGTA (F — Ca; N — Ca). For the measurements 22 μM actin and 0.5 μM lobster gelsolin-like protein were used. ▩, Native protein; ▨ recombinant protein.

headpiece domain do not compete for binding of actin, so the binding sites in actin for binding of gelsolin/villin segments 2 and 3 and the villin headpiece domain must be different.

Protein expression and biological activity of the recombinant protein

The fusion protein encoded on pLGS was expressed in *E. coli* and purified. As two proteins of almost the same size were recognized by the anti-(crustacean gelsolin antibody), it was necessary to investigate whether we had cloned the larger or the smaller protein. A comparison of recombinant protein with native muscle protein revealed that the fusion protein was approx. 5 kDa larger than the larger (molecular mass approx. 105 kDa) component of the native lobster protein (Figure 2, lanes D and E). As the fusion part is 47 amino acids long, this indicates that we have cloned the 105 kDa protein. There was already a weak band in the position of lobster gelsolin before induction, but samples of total cell protein after induction produced a prominent band co-migrating with native lobster gelsolin-like protein (Figure 6). Western-blot analysis showed that both these bands cross-reacted with the anti-(crustacean gelsolin) antibody (not shown), indicating weak but constitutive expression of this protein without induction and increased protein expression after induction. The constitutive expression is due to the *lac* promoter which controls the expression of the fusion protein. The fusion protein, synthesized to about 10% of total cell protein, was found in the fraction of inclusion bodies and was soluble in 8 M urea. On dialysis against buffer without urea, the renatured protein showed typical gelsolin activity (Figure 7) as revealed by viscometric assay of actin filament severing and nucleation of polymerization as well as of Ca^{2+}-dependence. The activities obtained for the recombinant fusion protein were comparable with those for the native protein. This indicates that the N-terminal fusion part of the protein does not significantly affect renaturing of protein activity.

The distribution of the 90 kDa actin-modulating proteins in the animal kingdom has not been extensively investigated outside the vertebrates. It is therefore of interest to assess where gelsolin-related proteins exist within the invertebrate phyla. The first example reported was a secretory gelsolin from *D. melanogaster* (Heintzelman et al., 1993). In contrast with Heintzelman et al. (1993) we know the tail muscle as the tissue origin for the actin-modulating protein in this study, and we have expressed this protein in *E. coli* to conform its basic biochemical characteristics as a gelsolin-like protein. In addition, we have demonstrated the biological activity of the recombinant protein after refolding, which is the prerequisite for further functional investigations both to localize the postulated third actin-binding site and to explain the complete EGTA-reversibility demonstrated for crustacean gelsolin–actin complexes (Bock et al., 1994), e.g. by single-domain expression and deletional mutagenesis.

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