Demonstration of at Least Two Different Actin-binding Sites in Villin, a Calcium-regulated Modulator of F-actin Organization*

John R. Glenney, Jr.,† Norbert Geisler, Phyllis Kaulfus, and Klaus Weber
From the Max-Planck-Institute for Biophysical Chemistry, D-3400 Göttingen, Federal Republic of Germany

Villin, one of the calcium regulated modulator proteins of F-actin organization, restricts F-actin to short filaments in the presence of calcium and bundles F-actin in the absence of calcium. Limited in vitro proteolysis of villin generates, in addition to a large core fragment (apparent Mr = 90,000) previously described, a small headpiece (Mr = 8,500). The finding that the F-actin nucleation and severing activity of villin, but not its bundling activity, is retained by the core suggested that the headpiece may be directly involved in bundling. Headpiece has now been purified and characterized. It shows strong F-actin binding both in the presence and absence of calcium, leading to a final stoichiometry of 1 headpiece to 1 F-actin monomer. Headpiece also inhibits villin-induced F-actin bundling: Thus villin expresses at least two distinct actin-binding sites localized on separate functional domains. Protein sequence analysis documents that the core comprises the NH$_2$-terminal portion of intact villin, whereas the headpiece covers the COOH-terminal 76 amino acids. We provide the amino acid sequence of the headpiece, which is currently the smallest F-actin binding peptide.

Microfilament assembly and turnover in the living cell seems to be under Ca$^{2+}$ control, regulated at least in part by a group of modulator proteins, often called F-actin-severing proteins (1-4). These proteins reveal strong Ca$^{2+}$ binding (5, 6) and confer Ca$^{2+}$ sensitivity on F-actin by restricting actin polymers to short filaments when the free Ca$^{2+}$ concentration reaches 10$^{-6}$ M (1, 2, 7). F-Actin severing proteins have been found in cell types as diverse as rabbit macrophages (3), Physarum polycephalum (2, 8), and chicken intestinal epithelial cells (1), and although ranging in molecular weight between 43,000 and 95,000, have so far revealed remarkably similar functions in the presence of Ca$^{2+}$ (4). One of these proteins, villin, isolated from chicken intestinal brush borders, has been recognized as a major structural protein of the highly ordered microvillus microfilament system (4).

Villin displays in vitro two distinct actin regulatory functions. First, at free Ca$^{2+}$ concentrations above micromolar levels, villin severs pre-existing F-actin and displays a concentration-dependent filament length restriction on actin assembly (1, 9-11). The latter mechanism seems to be due to the action of villin as a potent nucleation factor resulting filament capped at the "barbed" end (9). Second, at free Ca$^{2+}$ concentrations below 10$^{-7}$ M, relatively high concentrations of villin lead to the formation of tightly packed F-actin bundles similar in morphology to microvillus cytoskeletons and derivatives of these structures still containing villin (1, 5, 12, 13). The transition between these two opposed functions seems to be governed by the saturation of the Ca$^{2+}$ binding site, which is characterized by a micromolar dissociation constant (5). We have recently reported that mild in vitro proteolysis separates the bundling function from the severing function. Treatment of villin (Mr = 95,000) with Staphylococcus V-8 protease gave rise to a large core fragment (Mr = 90,000) which could be isolated by actin affinity chromatography (4, 9). The core retains the Ca$^{2+}$-dependent nucleation and severing activity of villin, but lacks the bundling activity observed with villin in the absence of Ca$^{2+}$ (4). Here we characterize for the first time the second proteolytic fragment, the headpiece, which is separated from core by actin affinity chromatography. We show that the headpiece covers the carboxyterminal 76 residues of the villin polypeptide chain, whereas the core is restricted to the NH$_2$-terminal part. The core and headpiece account for different structural and functional domains of the villin molecule. The headpiece binds to F-actin independently of Ca$^{2+}$ and acts as an inhibitor of villin-induced F-actin bundling. The combined results allow us to discuss the function of at least two distinct actin binding sites in the intact villin molecule.

MATERIALS AND METHODS

Isolation of Villin, Core, and Headpiece—Villin was isolated from chicken brush borders by our standard procedure (1, 9). Villin was digested with V-8 protease by two methods, each a slight modification of that described in a preceding paper (9). Procedure "A" was used to prepare digests which could be used directly in F-actin bundling assays without the need for dialysis or lyophilization. In this procedure, villin (6-12 mg/ml) in storage buffer consisting of 10 mM NaCl, 10 mM PIPES, 1 mM dithiothreitol, 3 mM NaN$_3$, 0.1 mM CaCl$_2$, pH 7.0, was digested with Staphylococcus V-8 protease (5 μg/ml) for 30 min at room temperature. V-8 protease was removed from the digest by affinity chromatography on monospecific rabbit anti-V-8 protease IgGs covalently bound to Sepharose 4B (1 mg of monospecific IgG/ml of settled gel) equilibrated in the storage buffer. The V-8 protease-depleted digest was then used directly to assay for actin-bundling activity. Alternatively, the solution was applied to a DNase-Sepharose column saturated with actin (9) and equilibrated with storage buffer. The flow-through from this column has been previously shown to contain only the villin headpiece without significant contamination by actin, villin, or villin core. Procedure "B" was employed for large scale preparation of villin headpiece to be used in actin binding assays and protein-chemical studies. Villin (7-10 mg/ml) was dialyzed against 50 mM ammonium bicarbonate containing 0.1 mM CaCl$_2$ and digested in this solution with 5 μg/ml of V-8 protease for 45 min at room temperature. V-8 protease was removed as before, and the solution was applied to a DNase-1 column saturated with actin and protein-chemical studies.

The abbreviations used are: PIPES, 1,4-piperazinediethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethly ether)-N,N,N',N'-tetraacetic acid; SDS, sodium dodecyl sulfate; dansyl, 5-dimethylaminonaphthalene 1-sulfonyl. 1

1 The abbreviations used are: PIPES, 1,4-piperazinediethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethly ether)-N,N,N',N'-tetraacetic acid; SDS, sodium dodecyl sulfate; dansyl, 5-dimethylaminonaphthalene 1-sulfonyl.
equilibrated in 50 mM ammonium bicarbonate, 0.1 mM CaCl₂. Column fractions were monitored by absorbance at 280 nm and the flow through fractions containing the headpiece were pooled and lyophilized. The lyophilized peptide was redissolved in distilled water, clarified by low speed centrifugation, and applied to a Sephadex G-50 column (7 x 1 cm) equilibrated in water. Peak fractions were pooled and aliquots were stored lyophilized, clarified by low speed centrifugation, and applied to a Sephadex G-150 column (1.5 x 55 cm) equilibrated in 0.8 M KCl, 10 mM PIPES, pH 7.0, in order to remove a small amount of 40,000-50,000-dalton fragments as described previously. The final preparation revealed only a single polypeptide with an apparent molecular weight of 90,000 when analyzed by SDS-polyacrylamide gel electrophoresis.

Low Speed Centrifugation Assay for F-actin Bundles—In order to study the bundling activity of villin by a biochemical assay, low speed centrifugation was used. This procedure, using villin-polypeptide bundles could be harvested by low speed centrifugation under conditions where normal F-actin remained in the supernatant. G-Actin at 0.5 mg/ml was incubated with varying amounts of the V-8 protease-depleted digest of villin (determined spectrophotometrically). The sample was adjusted to 50 mM KCl, 10 mM PIPES, 1 mM MgCl₂, 0.1 mM ATP, 0.1 mM dithiothreitol, 0.1 mM CaCl₂, 5 mM EGTA, pH 7.0, in a total volume of 200 µl. After incubation for 1 h at room temperature, the samples were centrifuged for 10 min at 15,000 g (Eppendorf centrifuge, model 5412). Supernatants were carefully separated from the pellets, and both fractions were adjusted to equal volumes with SDS sample buffer and boiled for 3 min. Samples were run on 7.5-20% polyacrylamide gradient SDS gels, stained with Coomassie blue, and quantitated by densitometry as described (14). Results were also monitored by electron microscopy. Pellets were fixed directly with 1% glutaraldehyde containing 0.2% tannic acid, embedded, and processed for sectional analysis as described (1).

F-actin Binding Assays—Villin, headpiece, or both were incubated in various ratios with actin in the buffer described above for the bundling assay, except that EGTA was excluded where indicated. After 1 h at room temperature, the samples were centrifuged for 20 min at 28,000 rpm (150,000 x g) in a Beckman Airfuge. Supernatants were separated from pellets, adjusted to equivalent volumes with SDS sample buffer, run on 7.5-20% polyacrylamide gradient SDS gels, and quantitated by densitometry.

Binding to Monomeric Actin—G-actin was centrifuged for 28 p.s.i. for 30 min in a Beckman Airfuge. The supernatant was mixed with headpiece (molar ratio, 2 actins to 1 headpiece), incubated for 1 h at room temperature in G-buffer (2 mM Tris, 0.2 mM dithiothreitol, 0.2 mM ATP, 0.2 mM CaCl₂, pH 7.6), and applied at 4°C to a Sephadex G-50 column (7 x 1 cm) equilibrated in the same buffer.

Miscellaneous Procedures—Actin was purified from bovine skeletal muscle by the method of Spudich and Watt (15), using the modification of MacLean-Fletcher and Pollard (16). Protein concentrations were determined spectrophotometrically using extinction coefficients of 13 cm⁻¹·mM⁻¹·cm⁻¹ for villin, 13 cm⁻¹·mM⁻¹·cm⁻¹ for the headpiece, and 6.6 cm⁻¹·mM⁻¹·cm⁻¹ for actin. In the case of headpiece, the value was monitored by amino acid analysis. Immunodiffusion was performed at room temperature using 1% agarose with 2% sodium acetate, embedded, and processed for sectional analysis as described.

Protein Chemical Techniques—NH₂-terminal sequence determinations of villin and core were performed using the SDS-dansyl technique of Weiner et al. (17). Preparative peptide maps of tryptic or thermolytic digests of the headpiece were obtained on Whatman 3MM paper as described (18). Peptides were detected and recovered by standard procedures and characterized by amino acid analysis and extended dansyl-Edman degradation. Amide assignments were based on the Offord plot and the results obtained by leucine aminopeptidase digestion (18). Digestion of villin, core, and headpiece by carboxypeptidase A (Sigma Chemical Co.) was performed in 0.1 M sodium acetate, pH 5.5, containing 4 mM urea. All other methods used are standard procedures and have been given elsewhere (18).

RESULTS

Isolation of Headpiece—In a previous report, we demonstrated that mild proteolysis of villin by V-8 protease yielded a core peptide of M₉ = 90,000. This cleavage is very specific as judged by one- and two-dimensional electrophoretic analysis (4). Subsequently, we were able to identify a further proteolytic fragment (M₉ = 8,000) which we call the headpiece. The initial products of V-8 digestion are the core and the headpiece (Fig. 1). Upon longer incubation times, further partial cleavage of the core into intermediate molecular weight peptides (M₉ = 40,000-50,000) is observed. Under these conditions, the headpiece becomes heterogeneous, and a closely spaced doublet is observed on SDS-polyacrylamide gels (see Ref. 9). In order to explore the biochemical properties of the headpiece, we used digestion conditions yielding the headpiece as a single band on SDS gels, even though villin was only incompletely converted to core (~90%) (see under “Materials and Methods”).

Since headpiece does not bind to actin immobilized on DNase-Sepharose, a simple purification scheme was devised. The flow-through from the DNase column was further subjected to gel filtration on Sephadex G-50. This preparation results in a good yield of homogeneous headpiece, which is salt-free and easily stored in lyophiliated form (Fig. 1). Villin core can be recovered from the DNase column by elution with EGTA (4, 9).

The headpiece is electrophoretically homogeneous and migrates on 20% acrylamide gels in the presence of SDS as a single band with an apparent molecular weight of approximately 8,000 (not shown). That headpiece provides major antigenic sites in the intact villin molecule is shown by immunodiffusion experiments using rabbit antiserum elicited with native intact villin (Fig. 2). No cross-reactivity with villin core is observed (Fig. 2), confirming the assumption that headpiece is not a secondary digestion product of villin core, but provides a separate domain of the villin molecule.

![Fig. 1](left) SDS-polyacrylamide gel electrophoresis of the isolated proteins used in this study. Lane A, intact villin; lane B, villin core; and lane C, villin headpiece. Villin and its core were run on 7.5-20% gradient gel. Gels were stained with Coomassie brilliant blue. The arrow indicates the position of the tracking dye.

![Fig. 2](right) Immunological reactivity of villin core and headpiece with rabbit antiserum elicited with native villin. Wells of the double diffusion plate were filled with: A, rabbit antiserum; B, intact villin; C, villin core; and D, villin headpiece. Note the lack of identity between the core and headpiece, but the partial identity between the core (or headpiece) and intact villin.
**F-actin Binding of Headpiece**—We have previously shown that isolated villin core does not bundle F-actin (4). It was therefore of interest to determine whether removal of headpiece is responsible for this loss of bundling. Use of anti-V-8 antibodies coupled to Sepharose enabled us to effectively remove the protease in these initial digests and to assay immediately for bundling activity. Since we found that villin-induced F-actin bundles could be harvested by low centrifugal forces under conditions where normal F-actin is not sedimented (Fig. 3), we used this semiquantitative assay. When villin was mixed with actin under bundling conditions, i.e. in the presence of EGTA, most of the actin became sedimentable when the molar ratio of villin to actin became higher than 1:10 (Fig. 4). When the same amount of V-8 protease-depleted digest of villin containing both core and headpiece was substituted, actin was not sedimented, confirming the loss of bundling activity by the cleavage itself.

Since villin core has been shown to retain the Ca$^{2+}$-dependent G-actin binding site(s) of intact villin (4, 9), we explored the possibility that the proteolytically derived headpiece may retain some biological activity to account for the loss of bundling. This is directly confirmed by the finding that headpiece binds to F-actin (Fig. 5; Table I) but not to G-actin. The headpiece can saturate F-actin close to a molar ratio of 1 headpiece/actin monomer. This binding is Ca$^{2+}$-independent and occurs equally well in the presence or absence of EGTA (Table I). As expected, high levels of headpiece inhibit the F-actin binding of intact villin, and high levels of villin (but not core) inhibit the binding of the headpiece (Table I).

When G-actin was mixed with headpiece and applied to a Sephadex G-50 column, all actin eluted at the void volume, and headpiece was observed as a single peak eluting at the position found for pure headpiece in separate experiments (data not shown).

**Morphology of Headpiece-F-actin Complexes**—When actin was mixed with subsaturating amounts of headpiece, the morphology of the resulting actin filaments appeared to be unchanged in negative stain analysis (Fig. 6A). As the headpiece level approaches saturating amounts (1 bound headpiece/actin), many filaments can be found which appear to be straighter or stiffer than normal F-actin (Fig. 6B). Although not all filaments in the latter preparations appear to be straight, normal F-actin can still be found as well, the long straight filaments are consistently found in the headpiece-treated samples and are absent in the controls. Cur-
Two Different Actin Sites in Villin

FIG. 6. Negative stain analysis of actin, villin, and headpiece mixtures. Actin (0.5 mg/ml) was mixed with headpiece at a molar ratio of either 1:1 (A) or 1:4 (B and D) with villin at 100 µg/ml (C and D). Polymerization was induced as described in the legend to Fig. 4. After 1 h at room temperature, samples were diluted 5-fold with polymerization buffer and immediately applied to a carbon-coated grid. Samples were stained with 2% aqueous uranyl acetate after brief fixation in 2% glutaraldehyde. Magnification, ×90,000. The bar in A is 0.11 µm. Note the straighter filaments with high concentrations of headpiece present (B and D). Note also the loss of villin-induced bundling (C) when the headpiece is included (D).

Table I

Co-sedimentation of actin, villin, and headpiece

Actin (0.5 mg/ml) was mixed at the specified molar ratio with villin, headpiece, or both, and adjusted to 50 mM KCl, 10 mM PIPES, 0.1 mM CaCl₂, 0.1 mM dithiothreitol, 0.1 mM ATP, 1 mM MgCl₂, pH 7.0, with or without 5 mM EGTA in a final volume of 0.1 ml. After incubation at room temperature for 1 h, F-actin filaments were harvested by centrifugation at 150,000 × g (28 p.s.i.) using a Beckman Airfuge. Supernatant and pellet fractions were adjusted to equivalent volumes of SDS sample buffer and analyzed on 7.5-20% polyacrylamide gels in the presence of SDS. Gels were stained with Coomassie brilliant blue, destained, and subjected to densitometry. The fraction of each component (expressed in per cent) present in the total pellet fraction was calculated.

| Proteins present | Ca²⁺ or EGTA excess | Percentage in high speed pellet |
|------------------|---------------------|-------------------------------|
| Actin alone      | EGTA                | 91                            |
| Headpiece alone  | EGTA                | 2                             |
| Actin:headpiece (1:2.5) | EGTA       | 34                            |
| Actin:headpiece (5:1) | EGTA       | 54                            |
| Actin:headpiece (5:1) | Ca²⁺       | 56                            |
| Actin:villin (10:1) | EGTA       | 30                            |
| Actin:villin:headpiece (10:1:2.5) | EGTA | 24                            |
| Actin:villin (2:1) | EGTA                | 79                            |
| Actin:villin:headpiece (10:1:0.4) | EGTA | 72                            |
| Actin:core:headpiece (2:1:0.4) | EGTA | 55                            |

Currently, the reason for these stiffer filaments is unknown. Actin-headpiece mixtures (Fig. 6, A and B) do not show the F-actin bundles induced by villin (Fig. 6C). When headpiece is added to actin-villin mixtures, bundling is strongly reduced, and bundles which are observed appear to be only loosely organized (Fig. 6D).

Protein-Chemical Characterization of Villin Headpiece—The amino acid composition (Table II) indicates the purity of the preparation. Assuming the presence of 1 residue each of histidine and methionine predicts the presence of approximately 75 amino acid residues. The approximate minimal molecular weight of 8,500 is in excellent agreement with the value of 8,000 estimated from SDS-polyacrylamide gels and Sephadex G-50 gel filtration data in the presence of 0.1 M KCl. For this molecular weight value, the amount of cysteine, isoleucine, and tyrosine is below the limit of detection, i.e. 0.05 residues/mol in our experiments. Thus, headpiece has a unique amino acid composition, because total villin contains tyrosine, isoleucine (1), and cysteine.² Dansylation detected only valine as NH₂-terminal residue. The COOH-terminal phenylalanine residue was identified by carboxypeptidase Y digestion (yield, 0.7 mol/mol). This experiment also predicted leucine as penultimate residue (see also below).

Two 1-mg preparations of headpiece were subjected to

² J. R. Glenney, Jr., N. Geisler, P. Kaulfus, and K. Weber, unpublished results.
proteolytic cleavage using either trypsin or thermolysin, respectively. The resulting peptides were separated by the two-dimensional preparative fingerprint system on Whatman 3MM paper (18). Peptides were detected by fluorescamine staining, recovered by elution, and characterized by amino acid composition calculated from the sequence (see Fig. 7).

The results complement our previous characterization of the COOH-terminal localization of the headpiece in the villin molecule, see text.

**Table II**

Amino acid composition of isolated villin headpiece and the composition calculated from the amino acid sequence (see Fig. 7).

| Amino Acid | A | B |
|------------|---|---|
| Aspartic acid | 8.6 | 9 |
| Threonine | 7.5 | 8 |
| Serine | 5.1 | 3 |
| Glutamic acid | 7.6 | 7 |
| Proline | 4.9 | 5 |
| Glycine | 3.3 | 3 |
| Alanine | 5.7 | 6 |
| Valine | 5.4 | 6 |
| Methionine | 1.1 | 1 |
| Isoleucine | ND | 0 |
| Leucine | 9.7 | 10 |
| Tyrosine | ND | 0 |
| Phenylalanine | 8.4 | 6 |
| Lysine | 6.8 | 7 |
| Histidine | 1.6 | 1 |
| Tryptophan | 0.8 | 1 |
| Cysteine | ND | 0 |

The headpiece provides the COOH-terminal domain of villin—Manual dansyl-Edman degradation in the presence of SDS (17) showed that villin and villin core have the same NH2-terminal sequence. Valine, glutamic acid or glutamine, and leucine are found in positions 1–3. Since headpiece shows the NH2-terminal sequence Val-Phe-Thr, the core fragment covers the NH2-terminal part of the villin polypeptide chain. Direct proof for the location of the headpiece at the COOH terminus of villin was obtained by the characterization of the COOH-terminal residue of villin and headpiece. Carboxypeptidase Y digestion indicated a leucine-phenylalanine sequence (final yield of phenylalanine, 0.8 mol/mol) for both villin and headpiece. In agreement with the placement of the core at the NH2 terminus and the glutamic acid specificity of V-8 protease, hydrazinolysis identified glutamic acid as the COOH-terminal end group in the core (yield, 0.3 mol/mol).

**Discussion**

Villin displays two structurally and functionally distinct domains separable by mild in vitro proteolysis with V-8 protease. Dansyl-Edman degradation and the determination of the COOH-terminal residues of villin and headpiece clearly show that the headpiece covers the COOH-terminal 76 amino acid residues of the villin polypeptide chain, whereas the core seems to span the remainder of the molecule, including the NH2 terminus. Currently, we don’t know whether core and headpiece are directly continuous, i.e. whether V-8 protease gives rise to a single proteolytic cut or if a further fragment(s) is generated, which is lost in the final preparation of core and headpiece. If the latter case were true, the putative further fragment(s) must be very small, considering the molecular weights of villin, core, and headpiece. Our protein-chemical studies document a unique amino acid sequence of the headpiece, curiously lacking cysteine, isoleucine, and tyrosine. The calculated molecular weight of headpiece is 8,536, making this fragment the smallest actin-binding protein.

The limited action of proteolytic enzymes on native proteins can be envisioned as a process in which certain flexible hinge regions connecting tightly packed functional domains are preferentially and specifically cleaved (see, for instance, Refs. 19 and 20). That the headpiece consists of a tightly packed domain is directly suggested by villin, core, and headpiece, whereas the core seems to span the remainder of the molecule, including the NH2 terminus. Currently, we don’t know whether core and headpiece are directly continuous, i.e. whether V-8 protease gives rise to a single proteolytic cut or if a further fragment(s) is generated, which is lost in the final preparation of core and headpiece. If the latter case were true, the putative further fragment(s) must be very small, considering the molecular weights of villin, core, and headpiece. Our protein-chemical studies document a unique amino acid sequence of the headpiece, curiously lacking cysteine, isoleucine, and tyrosine. The calculated molecular weight of headpiece is 8,536, making this fragment the smallest actin-binding protein.

Headpiece does not seem to bind to G-actin under our conditions, as indicated already by its isolation procedure. It does, however, bind to F-actin. The binding is independent of the presence or absence of Ca2+ and shows saturation at a molar ratio close to 1 headpiece for each actin monomer present in sedimentable F-actin form. This stoichiometry is obtained when actin and headpiece are mixed at a relative ratio of 1:4, and preliminary results indicate a dissociation constant in the micromolar range. Thus it is not surprising that headpiece inhibits villin-induced F-actin bundling observable in the absence of Ca2+ and that villin inhibits headpiece binding to F-actin under similar experimental conditions. The combined results indicate that headpiece reveals one of the F-actin-binding sites responsible for the F-actin-bundling activity of villin observed in the absence of Ca2+.

The results complement our previous characterization of...
the biological properties of the villin core (4, 9). This large fragment retains the Ca\(^{2+}\)-dependent properties previously documented for villin. The core shows severing of preformed F-actin and restricts, in a concentration-dependent manner, the length of F-actin filaments, when added to G-actin before salt-induced polymerization. This phenomenon is probably explained by the potent nucleation activity of villin or its core on actin assembly in the presence of Ca\(^{2+}\), since villin core caps the barbed end leading to a unidirectional elongation toward the pointed end. Consequently, treadmilling of actin monomers through the resulting filaments is inhibited (9).

The final result, directly governed by the molar ratio of actin to villin, is the expression of more and necessarily shorter actin assembly of EGTA, we prefer the conclusion that upon depletion of Ca\(^{2+}\), a conformational change allows the transition of the villin into core and headpiece together with the unique amino acid sequence of the headpiece clearly show that the two isoforms are closely related. The difference in isoelectric points resides in the core (4) and may either be due to local sequence heterogeneity or, more likely, to a partial posttranslational modification, such as, for instance, a phosphorylation. The possible physiological importance of this heterogeneity remains to be elucidated.

Acknowledgments—We gratefully acknowledge Dr. M. Osborn for anti-villin antisera and U. Pleissmann for excellent technical assistance.

REFERENCES

1. Bretscher, A., and Weber, K. (1980) Cell 20, 839–847
2. Hasegawa, T., Tokayashi, S., Hayashi, R., and Hatano, S. (1980) Biochemistry 19, 2677–2683
3. Yin, H. L., and Stossel, T. P. (1979) Nature 281, 583–585
4. Glenney, J. R., Jr., and Weber, K. (1981) Proc. Natl. Acad. Sci. U.S.A., in press
5. Glenney, J. R., Jr., Bretscher, A., and Weber, K. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 6458–6462
6. Yin, H. L., and Stossel, T. P. (1980) J. Biol. Chem. 255, 9490–9495
7. Yin, H. L., Zaner, K. S., and Stossel, T. P. (1980) J. Biol. Chem. 255, 9494–9500
8. Hinsen, H. (1981) Eur. J. Cell Biol. 23, 225–233
9. Glenney, J. R., Jr., Kauflus, P., and Weber, K. (1981) Cell 24, 471–480
10. Mosekler, M. S., Graves, T. A., Whiton, K. A., Falco, N., and Kauflus, P. (1980) J. Cell Biol. 87, 809–822
11. Craig, S. W., and Powell, L. D. (1980) Cell 22, 739–746
12. Matsudaira, P. T., and Burgess, D. R. (1979) J. Cell Biol. 83, 667–673
13. Matsudaira, P. T., and Burgess, D. R. (1980) J. Cell Biol. 87, 221
14. Glenney, J. R., Jr., and Weber, K. (1980) J. Biochem. 255, 10551–10554
15. Spudich, J. A., and Watt, S. (1971) J. Biochem. 245, 4860–4871
16. MacLean-Fletcher, S., and Pollard, T. D. (1980) Cell 329, 431
17. Weis, A. M., Platt, T., and Weber, K. (1972) J. Biol. Chem. 247, 3242–3251
18. Geisler, N., and Weber, K. (1980) Eur. J. Biochem. 111, 425–433
19. Neurath, H. (1980) in Protein Folding (Jaenicke, R., ed) p. 501, Elsevier, North Holland, Amsterdam
20. Geisler, N., and Weber, K. (1977) Biochemistry 16, 938–943