Group-Specific Component (Vitamin D Binding Protein) Prevents the Interaction between G-Actin and Profilin†

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ABSTRACT: Profilin purified from human platelets formed a 1:1 molar ratio complex with rabbit skeletal muscle G-actin but was displaced by purified serum Gc (vitamin D binding protein) in a dose-dependent fashion as assessed by chromatography and ultrafiltration. This suggested that Gc and profilin competed for the same binding area on G-actin, with Gc–G-actin complexes being more stable than profilin–G-actin complexes in vitro. The binding domain for Gc on G-actin was localized to a 16 000-Da C-terminal fragment of G-actin generated by *Staphylococcus aureus* V8 protease, as judged by comigration on two-dimensional electrophoresis and also by overlaying electrophoresis gels with ^{125}I-Gc. Previous studies have reported that residues 374 and 375 of G-actin are essential for binding of profilin. In this study, experiments involving tryptic removal of Cys-374 labeled with the fluorescent probe *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)-ethylenediamine showed that these C-terminal amino acids were not necessary for interaction with Gc.

The central role played by the reversible polymerization and cross-linking of actin in the structure, configuration, and motile activities of nonmuscle cells is broadly accepted, but the mechanisms that regulate these phenomena are not yet fully understood (Korn, 1982). The presence of 50% or more of total actin in the monomeric G form in many nonmuscle cells is probably the result of interactions between monomer and G-actin-sequestering proteins that inhibit polymerization (Korn, 1982; Craig & Pollard, 1982). One such protein is profilin, a low molecular weight, basic protein that is widely distributed in tissues of both vetebrates and nonvertebrate organisms. This protein undergoes a high-affinity interaction with G-actin with stoichiometry of 1:1, to form a complex, profilactin (Carlsson et al., 1977; Chien-Hua Tseng & Pollard, 1982; Harris & Weeds, 1978; Ozaki & Hatano, 1984; Reichstein & Korn, 1979; Tilney, 1976; Tobacman & Korn, 1982). These observations have led to the proposal of several mechanisms that might regulate actin polymerization. For example, cell stimulation in vivo appears to lead to dissociation of profilactin complexes with subsequent nucleation and polymerization of actin to generate filaments (Blikstad et al., 1980; Grumet & Lin, S., 1980; Lassing & Lindberg, 1985; Malm et al., 1980). Recent evidence obtained in vitro suggests the possible involvement of membrane phospholipids in these phenomena (Lassing & Lindberg, 1985).

Another sequestering protein is Gc[1] or vitamin D binding protein, which also binds G-actin with high affinity and stoichiometry of 1:1 (Chen et al., 1979; Daiger et al., 1975; Haddad, 1982; Putnam, 1977; Van Baelen et al., 1980). This protein was originally described in the circulation but has also been demonstrated to be present in the cytoplasm of nucleated cells and more recently in relation to the surface membrance of circulating mononuclear cells (Cooke et al., 1979a,b; Galbraith et al., 1985; Petrini et al., 1983, 1984, 1985). On T cells, surface Gc appears to become spatially associated with several membrane components, including receptors for the Fc portion of IgG (Galbraith et al., 1985; Petrini et al., 1985). On B lymphocytes, surface Gc codistributes into patches and caps with membrane immunoglobulin (MIg) when such cells are activated by complexing of MIg with specific antibody as ligand (Petrini et al., 1983). The latter observation is consistent with a role for Gc in signal transduction, and further evidence for this has recently been obtained in the finding that in pancreatic acinar cells Gc appears to be a major substrate for Ca^{2+}/phospholipid-dependent protein kinase or C-kinase (Wooten et al., 1985).

In view of the ubiquitous distribution of both profilin and Gc and their potential importance in the ordering and regulation of cellular actin, we have performed studies to clarify the interactions of these two ligands with G-actin. The results demonstrate that binding of Gc to G-actin prevents interaction of the latter with profilin, suggesting that Gc and profilin may compete for a common binding site on G-actin. This domain was localized to a 16 000-Da C-terminal fragment of actin. However, although the two terminal residues of G-actin have been reported to play a crucial role in interactions with profilin, this appeared not to be the case for Gc.

Experimental Procedures

Protein Purification and Labeling. Human Gc and rabbit G-actin were purified as previously described, and protein

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[1] Abbreviations: DNase, deoxyribonuclease I (3.1.21.1); DTT, dithiothreitol; 2D electrophoresis, two-dimensional electrophoresis; Gc, group-specific component; IAEDANS, *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine; IEF, thin-layer analytical isoelectric focusing; ^{125}I-Gc, ^{125}I-radioiodinated Gc; NaDodSO$_4$–polyacrylamide gel electrophoresis, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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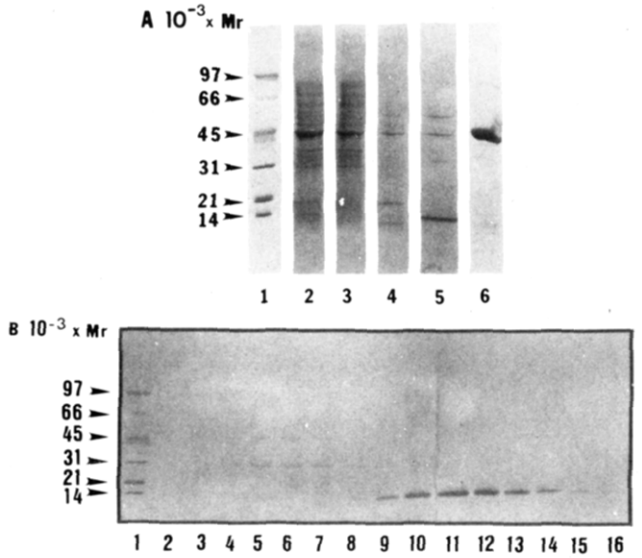

FIGURE 1: Purification of profilin. Supernatants from sonicated human platelets were chromatographed sequentially on a DNase affinity column and a Bio-Gel P30 column as described in text. The fractions were submitted to $NaDodSO_4$–polyacrylamide gel (5–20%) electrophoresis with Coomassie Blue staining. (a) Protein content of representative fractions of a DNase column: lane 1, low molecular weight standards; lane 2, starting sample; lane 3, unbound proteins eluted with G buffer; lane 4, bound protein eluted with 0.2 M NaCl in G buffer; lane 5, protein eluted with KI (1 M) in G buffer; lane 6, protein eluted with a buffer containing 3 M guanidine hydrochloride. (b) Fractions eluted with KI pooled, concentrated, and chromatographed on Bio-Gel P30: lane 1, low molecular weight standards; lanes 2–16, fractions eluted from the Bio-Gel P30 column. Note that lanes 9–16 contain only one major species of 15000 Da, which appeared as a doublet. These fractions were pooled, concentrated, and used for further studies.

concentrations of the final preparations were measured (Chapuis-Cellier et al., 1983; Goldschmidt-Clermont et al., 1985a). Cysteine-374 of G-actin was labeled with the dye *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine (IAEDANS) according to methods described by Takashi (1979). Gc was labeled with ^{125}I as previously described (Goldschmidt-Clermont et al., 1985a). Profilin was prepared from human platelets according to Kobayashi et al. (1982), with minor modifications. Pooled platelets (4.3×10^{11}) were isolated from peripheral blood of normal volunteers, washed, and resuspended in a buffer containing 5 mM Tris, pH 7.5, 0.1 mM ATP, 0.1 mM $CaCl_2$, and 0.5 mM DTT (G buffer). Cells were sonicated twice for 10 s at 4 °C and centrifuged at 12000*g* for 5 min, and supernantants were harvested. The final protein concentration was 10 mg/mL. Five milliliters of supernatant was loaded onto an affinity column of immobilized DNase (Cooper Biomedical, Malvern, PA) containing 0.12 g of DNase. The column was washed sequentially with 100 mL of G buffer and 100 mL of 0.2 M NaCl in G buffer, and profilin was then eluted with 1.0 M potassium iodide (KI) in G buffer (Figure 1a, lane 5). The remaining actin and other proteins were then stripped with 0.5 M sodium acetate, 1 mM $CaCl_2$, 30% glycerol, and 3.0 M guanidine hydrochloride (pH 6.5) (Figure 1a, lane 6). The column was regenerated by washing with G buffer (200 mL). The KI eluate representing crude profilin was concentrated on a Centricon microconcentrator, 10000-Da cutoff (Amicon, Danvers, MA), and further purified by chromatography over a Bio-Gel P30 (Bio-Rad Laboratories, Richmond, CA) column (5 × 170 mm, flow rate 3 mL/h, 0.3-mL fractions, 22 °C). Fractions were analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis. Those containing profilin, which appeared as a 15000-Da doublet as previously described (Malm et al., 1983), were pooled and concentrated (Figure 1b, lanes 9–16).

Proteolytic degradation of G-actin was performed with trypsin (Sigma), *Staphylococcus aureus* V8 protein (Pierce), or carboxypeptidase A (Sigma) as described previously (Mornet & Ue, 1984; Goldschmidt-Clermont et al., 1986) in G buffer at 22 °C, with protein to enzyme ratios of 3:1 to 10:1 as specified in the Results.

Chromatographic Separation. Gel filtration was also performed in prepacked PD-10 columns (7.5 × 50 mm, 1 mL/min, 1-mL fractions, 22 °C) containing Sephadex G-25 (Pharmacia Fine Chemicals) to separate IAEDANS bound to G-actin from free label, before and after enzymatic proteolysis. Absorbance at 280 nm was used throughout to record the optical density profile.

Electrophoretic Procedures. Gradient (5–20%) $NaDodSO_4$–polyacrylamide gel electrophoresis (Laemmli, 1970), thin-layer analytical isoelectric focusing (IEF; Emerson et al., 1984), and two-dimensional (2D) electrophoresis (Goldschmidt-Clermont et al., 1985b) were performed as reported. Gels were stained with Coomassie blue or silver (Merril et al., 1981).

Fluorescence Measurements. These were performed with an Aminco-Bowman spectrophotofluorimeter equipped with a ratio photometer. For IAEDANS, emission was measured at 470 nm with excitation at 340 nm, at 22 °C (Takashi, 1979).

Actin Polymerization Assay. This involved measurement of the amount of actin pelleted after centrifugation as described previously (Harris et al., 1980; Thorstensson et al., 1982; Yonezawa et al., 1985). Experiments were performed in cellulose proprionate centrifuge tubes (Beckman) with micro amounts of protein as indicated. Proteins were incubated in G buffer supplemented with 0.1 M KCl and 1 mM $MgCl_2$ for 30 min at 22 °C and then centrifuged at 100000*g* for 30 min at 22 °C. The supernatants and pellets were subjected to $NaDodSO_4$–polyacrylamide gel electrophoresis and analyzed after Coomassie Brillant Blue staining by computerized scanning as previously described (Goldschmidt-Clermont et al., 1986).

Onlay with ^{125}I-Gc. This assay was performed with ^{125}I-Gc (sp act. 2–3 μCi/μg) within 24 h of labeling. Native IAEDANS–actin or proteolytic fragments thereof were run on $NaDodSO_4$–polyacrylamide gel electrophoresis and electroblotted onto nitrocellulose (Towbin et al., 1978). After incubation for 30 min in buffer (20 mM Tris and 0.5 M NaCl, pH 7.5) containing 1.5% gelatin (Hipure, Norland Products Inc., New Brunswick, NJ), the paper was reacted with 100 μCi of ^{125}I-Gc diluted in 50 mL of G buffer for 60 min at 22 °C, washed three times with G buffer for 20 min, and autoradiographed.

Ultrafiltration of Proteins. Aliquots of G-actin (2.5 μg) were reacted with profilin (2.0 μg) for 30 min at 22 °C in G buffer. Increasing quantities of Gc (0.45, 0.9, 1.8, 3.6, 7.2 μg) were then added to the mixture and incubated at 22 °C for 15 min. Each aliquot (30 μL) was diluted with 500 μL of G buffer and centrifuged in Centricon Microconcentrators (30000-Da cutoff, 5000*g* for 30 min at 4 °C). Concentrated protein in the retentate was analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis and computerized densitometric scanning as previously described (Goldschmidt-Clermont et al., 1986).

RESULTS

Interactions between G-Actin, Profilin, and Gc. The ability of purified profilin to interact with actin was monitored with

Table I: Quantitation of Sequestering Effect of Gc and Profilin on G-Actin[a]

mixture	supernatant [units (%)]	pellet [units (%)]
actin in G buffer	680 (93)	52 (7)
actin in F buffer	23 (4)	514 (96)
actin + Gc in F buffer	480 (97)	16 (3)
$actin_{CA}$ in F buffer	299 (42)	420 (58)
$actin_{CA}$ + Gc in F buffer	843 (95)	40 (5)
actin + $profilin_1$ in F buffer	464 (81)	106 (19)
actin + $profilin_2$ in F buffer	573 (90)	65 (10)

[a] The data presented correspond to the intensity (arbitrary units) of the 42 000-Da actin band (mean of two experiments) measured by computerized densitometric scans of 5–20% continuous-gradient Na-$DodSO_4$–polyacrylamide gel electrophoresis. Each starting sample contained actin (7 μg), with or without addition of Gc (8 μg) or profilin ($profilin_1$, 1.9 μg; $profilin_2$, 3.9 μg). For certain experiments involving Gc, actin was first treated with carboxypeptidase ($actin_{CA}$). Pelleting assays were performed as described in the text.

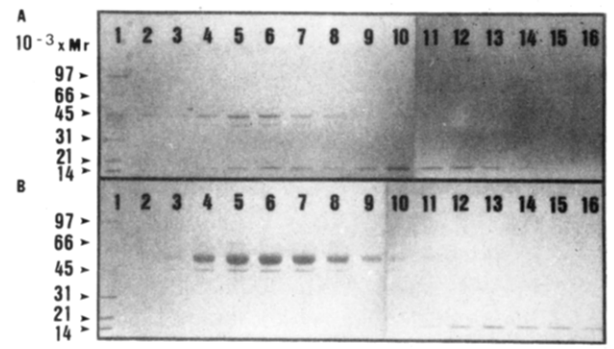

FIGURE 2: Formation of profilactin complexes in vitro and interaction with Gc. Profilin (100 μg) was mixed with equimolar quantities of G-actin (350 μg) and rechromatographed on the Bio-Gel P30 column (a) in the absence of Gc and (b) after incubation for 150 min with a molar excess of Gc to G-actin (1.5:1). Note in the case of G-actin–profilin mixture the comigration of >50% of profilin with G-actin in the void volume (a, lanes 2–9), whereas in the presence of Gc all profilin eluted in the included fractions (b, lanes 11–16).

two different approaches. First, the ability of such preparations to inhibit polymerization of G-actin was checked by pelleting assays. When G-actin only was exposed to 0.1 M KCl and 1 mM $MgCl_2$, >90% of actin subsequently pelleted, as judged densitometrically (Table I). In contrast, when saturating amounts of profilin were present, >90% of actin remained unpelleted (Table I). Second, the ability to bind G-actin was examined by chromatography of an equimolar mixture of G-actin and profilin over the same Bio-Gel P30 column used for purification of profilin. In contrast to the profile observed for the latter, a large amount of 15 000-Da material (ca. 50%) eluted earlier in the void volume with a densitometric profile identical to that of G-actin (Figure 2a, lanes 2–8). Together, these data confirmed that platelet profilin obtained by this means retained the ability to interact with stoichiometry of 1:1 with purified G-actin, even though the presence of 15 000-Da material in the included volume (Figure 2a, lanes 9–16) indicated that the reconstituted profilactin complexes were not entirely stable, a point noted previously (Lal & Korn, 1985; Malm et al., 1983; Reichenstein & Korn, 1979).

The next experiments were designed to determine whether a ternary complex could be formed between Gc, G-actin, and profilin by chromatography of G-actin and profilin on Bio-Gel P30 as above, but with addition of Gc. For these experiments, profilin (100 μg) and G-actin (325 μg) were first mixed together for 30 min at 4 °C to allow formation of profilactin complexes, and then Gc (450 μg) was added and the mixture incubated for 150 min at 4 °C prior to chromatography. In

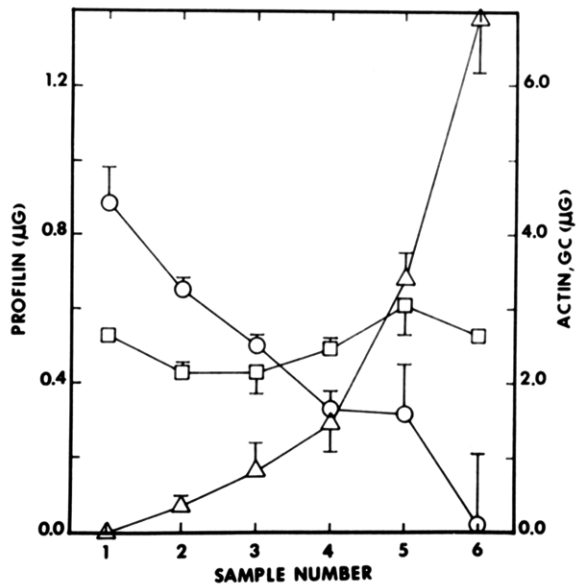

FIGURE 3: Ultrafiltration of profilin and G-actin, in the presence and absence of Gc. Each mixture was centrifuged in Centricon filters (cutoff 30 000 Da) as described in the text. Profilin, G-actin, and Gc were quantitated in the retentate by computerized densitometric scanning of the 15 000-, 43 000-, and 56 000-Da bands seen on Na-$DodSO_4$–polyacrylamide gel electrophoresis: ○, profilin; □, G-actin; △, Gc. The results shown correspond to mean ● SD of two experiments. In the absence of G-actin only small amounts of profilin (<15%) remained in the retentate, and all subsequent results were corrected to reflect this, whereas in the presence of saturating quantities of G-actin >95% of prfilin was retained. Addition of increasing quantities of Gc led to a corresponding increase in the quantities measured in the retentate and a reciprocal loss of profilin, without any significant change in the quantities of G-actin present.

contrast to results obtained in the absence of Gc where some profilin coeluted with G-actin in the void volume (Figure 2a, lanes 2–9), the addition of Gc resulted in the appearance of profilin only in the included volume (Figure 2b, lanes 11–16). With a slight excess of Gc over G-actin (molar ratio 1.5:1), displacement was apparently complete with no detectable profilin in the excluded fractions containing actin (Figure 2b, lanes 2–10).

This observation indicating dissociation of profilactin complexes by Gc was further investigated by ultrafiltration of proteins on Centricon microconcentrators (cutoff 30 000 Da), with Na-$DodSO_4$–polyacrylamide gel electrophoresis of the retentate and densitometric scanning. When preparations of profilin were filtered in the absence of G-actin, <15% of the 15 000-Da band corresponding to profilin remained in the retentate, whereas in the presence of G-actin equimolar amounts of profilin remained in the retentate. Addition of increasing amounts of Gc to such profilactin complexes then caused a dose-dependent decrease in profilin in the retentate; at Gc to G-actin ratios of 0.5:1 and 2:1, respectively, 50% and >99% of profilin were displaced (Figure 3).

A possible ternary complex between profilin, G-actin, and Gc was also sought by means of the 2D electrophoretic method used previously to demonstrate formation of ternary complexes between DNase, G-actin, and Gc (Goldschmidt-Clermont et al., 1985b). Although Gc and G-actin clearly comigrated in the first dimension, in no instance was profilin found in association with this complex (Figure 4). This finding was unaffected by the order of addition of components, e.g. profilin being added before, simultaneously with, or after Gc. The data collectively indicate that Gc and profilin did not interact simultaneously with G-actin and indeed may have competed for the same area of the molecule.

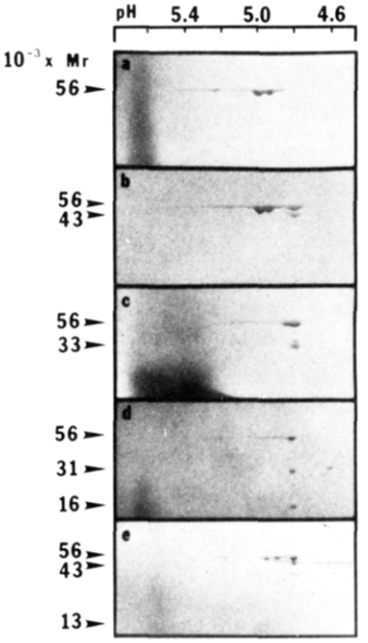

FIGURE 4: Two-dimensional electrophoresis of Gc, Gc–G-actin, and Gc–G-actin fragments. Isoelectric focusing (pH 4–6) and NaDodSO$_4$–polyacrylamide gel electrophoresis (5–20%) were performed as described. For clarity, Gc_{1-1} phenotype has been used in all experiments illustrated. Similar results were obtained with the other major phenotypes, Gc_{1-2} and Gc_{2-2}: (a) 8 μg of purified Gc, with two spots of 56 000 Da, at pH 4.97 and 4.92 corresponding to Gc_{1-1} slow and Gc_{1-1} fast, respectively; (b) 8 μg of Gc and 4 μg of G-actin with a 56 000-Da spot corresponding to the excess of Gc over actin and anodal spots of 56 000 and 43 000 Da at pH 4.80, typical of Gc–G-actin complexes; (c) 10 μg of purified complexes of Gc and the 33 000-Da fragment of G-actin prepared as described in the text. [Note comigration of the 56 000-Da species with the 33 000-Da fragment of G-actin in the first dimension (pH 4.80)]; (d) 6 μg of Gc and 4 μg of G-actin treated by V8 protease (protein to enzyme molar ratio of 3:1) [note comigration of the 56 000-Da species with fragments of G-actin (30 000 and 16 000 Da) at pH 4.80; note also the prsence of the spot 32 000 Da, pH 4.60, which appears to correspond to V8 protease]; (e) 4 μg of Gc, 3 μg of G-actin, and 3 μg of profilin. The appearances are similar to those of b, with no additional low molecular weight spot corresponding to profilin migrating with Gc–G-actin complexes.

Binding of Gc to V8 Protease Fragments of G-Actin. On the basis of previous studies, the C-terminal end of G-actin appears to be the major site of interaction with profilin, particularly at the penultimate and C-terminal residues (Lal & Korn, 1985; Malm, 1984; Malm et al., 1980, 1983). Experiments were performed to determine whether Gc interacts with the same or closely related domain of G-actin. For this purpose, we used G-actin labeled with IAEDANS, since this probe is specifically linked to cysteine-374 at the C-terminal end (Mornet & Ue, 1984; Takashi, 1979).

Certain information concerning the profile of enzymatic degradation of G-actin by *S. aureus* V8 protease has been recently described. Thus, the major breakdown product of G-actin with V8 protease appears to be a 16 000-Da C-terminal fragment (Mornet & Ue, 1984; Johnson et al., 1979). For the experiments described below, proteolysis of IAEDANS–actin by *S. aureus* V8 protease was performed at 22 °C for 120 min (protein to enzyme ratio of 3:1). Mixtures of Gc and G-actin fragments were examined by 2D electrophoresis. This demonstrated comigration of Gc with breakdown products of G-actin in the first dimension, the smallest corresponding to the fluorescent 16 000-Da C-terminal fragment of IAEDANS–actin (Figure 4d). Binding occurred regardless of whether or not actin was cleaved before or after complex formation with Gc. Under the conditions described, no degradation of Gc by V8 protease was evident. Further evidence of such interaction was obtained from the observation that interactions of Gc with actin fragments led to an anodal shift of the isoelectric point of the resulting complexes (pH 4.80) indistinguishable from that observed after interaction between Gc and intact G-actin (Emerson et al., 1984).

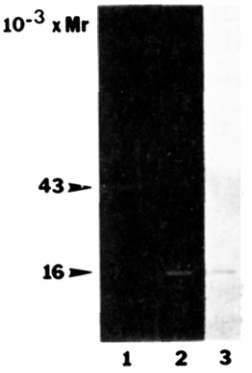

FIGURE 5: NaDodSO$_4$–PAGE of proteolytic actin fragments and ^{125}I-Gc overlay. V8 protease treatment of IAEDANS–actin was performed as described in the text (protein to enzyme ratio of 3:1), and samples were submitted to SDS-PAGE and transblotting onto nitrocellulose: (1 and 2) intact actin (5 μg) and 16 000-Da fragment (from 5 μg of actin), examined by fluorescent light; (3) transblot of lane 2 with ^{125}I-Gc overlay and autoradiography. Note binding of ^{125}I-Gc to the 16 000-Da fragment.

Binding of Gc to the fluorescent 16 000-Da fragment of G-actin was also sought by ^{125}I-Gc overlay. The position of the native IAEDANS–actin and the 16 000-Da fragments on nitrocellulose was easily identified under UV light. Autoradiography showed a radiolabeled band that corresponded to the fluorescent 16 000-Da fragment (Figure 5), but none of the other proteolytic fragments obtained with V8 protease bound detectable amounts of ^{125}I-Gc.

Enzymatic Removal of G-Actin C-Terminal Residues. Similar experiments were performed with trypsin. This enzyme generates a large proteolytic C-terminal fragment of 33 000 Da corresponding to residues 69–372, indicating the presence of two major tryptic cleavage sites on G-actin, one at lysine-69 and the other at lysine-373 (Jacobson & Rosenbusch, 1976; Mornet & Ue, 1984). We reported earlier that Gc forms a 1:1 molar ratio complex with this major tryptic fragment of actin. Furthermore, this interaction provided reciprocal protection for both proteins against further proteolysis, suggesting that the appropriate interaction occurred at some point between residues 69 and 372 (Goldschmidt-Clermont et al., 1986). However, in these experiments, removal of the C-terminal residues was not monitored. Since the penultimate and C-terminal residues of G-actin were reported to be crucial for interaction with profilin (Lal & Korn, 1985; Malm, 1984; Malm et al., 1980, 1983), we further studied their role in binding of G-actin to Gc.

Tryptic removal of residues 374 and 375 was monitored by measurement of the fluorescence of the fragment obtained, in the presence or absence of Gc. Labeled G-actin alone (80 μg), or together with an approximately equimolar amount of Gc (100 μg), was treated for 1 h with trypsin (protein to enzyme ratio of 5:1), at 22 °C, and then filtered through a PD-10 column. Under these conditions, >90% of native G-actin was converted into the 33 000-Da fragment, and <10% of native Gc was degraded, as reported (Goldschmidt-Clermont et al., 1986). The fractions (1 mL) were then analyzed for IAEDANS fluorescence and also by NaDodSO$_4$–polyacrylamide gel electrophoresis for their protein content. This

showed that after proteolysis the fluorescent probe was shifted from the G-actin protein peak (fractions 3 and 4) to a low molecular weight peak (fractions 7–11), indicating enzymatic removal of residues 374 and 375. Moreover, enzymatic cleavage occurred regardless of the presence or absence of added Gc, demonstrating a failure of the latter to protect this trypsin proteolytic site on G-actin.

Analysis of the same fractions by $NaDodSO_4$–polyacrylamide gel electrophoresis confirmed conversion of G-actin to the 33 000-Da fragment in the protein peak, indicating that removal of the labeled probe was specific and not simply due to complete degradation of G-actin. In the case of Gc–G-actin complexes, treatment with trypsin resulted in the presence of unaltered 56 000-Da native Gc in addition to the 33 000-Da fragment. When the gel was studied under UV light, the 33 000-Da fragment was no longer fluorescent. Moreover, binding of Gc to the 33 000-Da fragment under these circumstances was confirmed by their comigration in the first dimension of 2D electrophoresis (Figure 4c). These results collectively suggested that the two C-terminal residues are not necessary for binding of Gc to the 33 000-Da fragment. This observation was further investigated by treatment of G-actin with carboxypeptidase (in G buffer at 22 °C for 60 min and a protein to enzyme ratio of 10:1) and examination of the ability of Gc to prevent filament formation by pelleting assays. Although carboxypeptidase treatment of G-actin did not prevent subsequent polymerization as noted previously (Malm, 1980), the addition of Gc still caused inhibition of this process (Table I).

Discussion

In this report, we present evidence that Gc competed with profilin for binding to G-actin and that interactions between G-actin and Gc appeared to be more stable than those between profilin and G-actin. These results are consistent with the higher reported affinity of Gc for G-actin [$K_a \approx 2 \times 10^8$ M^{-1} (Goldschmidt-Clermont et al., 1985b)] than that of profilin [$K_a \approx 2 \times 10^5$ M^{-1} (Lal & Korn, 1985)]. However, this observation should be tempered by the possibility that profilactin complexes reconstituted in vitro after purification may be less stable than those formed under native conditions in vivo. It has been reported for example that additional protien(s) or other cofactor(s) may be necessary to stabilize profilactin complexes in vitro (Malm et al., 1983), whereas artificially constituted complexes between purified Gc and G-actin are relatively stable and easily isolated by a number of techniques including gel filtration and 2D electrophoresis (Goldschmidt-Clermont et al., 1985b, 1986). It is of interest that Gc was able in vitro to displace profilin from G-actin. The relevance of this observation in vivo will require further investigation, although it has previously been suggested that interactions of G-actin with both Gc and profilin are similar in functional terms (Coue et al., 1984). On the other hand, their respective binding sites on G-actin may not be exactly homologous, since the last two C-terminal residues of G-actin have been reported to be essential for the binding of vertebrate and Acanthameba profilin (Lal & Korn, 1985; Malm, 1984; Malm et al., 1980, 1983) whereas they do not seem to be important components of the binding domain for Gc.

We identified a 16 000-Da segment of G-actin responsible for interaction with Gc, which is apparently located at the C-terminal end of the molecule, since it includes the fluorescent probe IAEDANS that labels Cys-374 (Mornet & Ue, 1984; Takashi, 1979). These data are therefore consistent with our previous reports indicating that the binding site for Gc is distinct from that of DNase I (Goldschmidt-Clermont et al., 1985b, 1986). The latter is known to interact with a segment of the molecule at the N-terminal end, including residues Lys-50, Lys-61, Lys-68, Tyr-53, and Tyr-69 (Burtnick & Chan, 1980a,b; Sutoh, 1984).

Another interesting observation is the alteration in isoelectric point of Gc bands that resulted from interaction with G-actin treated with V8 protease (Figure 4). The extent of shift was indistinguishable from that noted previously for interaction of Gc with native G-actin (Emerson et al., 1984). This suggested that the specific physicochemical consequences of interaction between Gc and G-actin may result from configurational changes occuring on Gc following interaction with a specific domain of G-actin. A change in confirguration of Gc has also been reported upon interaction of Gc with 25-hydroxycholecalciferol (Surarit & Svasti, 1980).

The respective biological roles of profilin and Gc are under intensive investigation. Existing evidence indicates the presence of a relatively high concentration of profilactin complexes underneath the cell membrane; these act as a pool of monomeric actin for generation of new filaments (Harris & Weeds, 1978; Lindberg et al., 1981; Markey et al., 1981; Sundqvist & Ernst, 1976; Tilney, 1975). Cell stimulation then results in a decrease in this precursor pool (Lindberg et al., 1981). Moreover, an interaction between phosphatidylinositol and profilin, which leads to actin polymerization has recently been described in vitro (Lassing & Lindberg, 1985). This finding suggests a possible link between induction of actin filament formation and phospholipid metabolism following ligand membrane receptor interactions, and it is important to note that these are two crucial phenomena following cell activation (Lindberg et al., 1981; Nishizuka, 1984).

On the other hand, Gc, seems to be implicated in the mobility of membrane immunoglobulin on the surface of B cells (Petrini et al., 1983), a phenomenon that could be related to its ability to interact with G-actin. Moreover, in rat pancreatic acinar cells, Gc is a major substrate for Ca^{2+}/phospholipid-dependent protein kinase (C-kinase) (Wooten et al., 1983). Since profilin concentrations may be higher than those of Gc within cells, it was considered important in the present study to further confirm the possibility of interactions between Gc and G-actin inside cells, even in the presence of other major G-actin-sequestering proteins such as profilin. The observation of an apparently specific binding site on G-actin, and the stability of interactions between Gc and G-actin regardless of the presence of saturating amounts of profilin, indicate the possibility of a crucial role played by Gc in cell biology.

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