

# Mutations in $\beta$ -actin: influence on polymer formation and on interactions with myosin and profilin

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Two  $\beta$ -actin mutants, one with proline 38 replaced with alanine (P38A) and the other with cysteine-374 replaced with serine (C374S), as well as the wild-type  $\beta$ -actin, were expressed in the yeast, *S. cerevisiae*, purified to homogeneity, and analyzed in vitro for polymerizability and interaction with DNase I, myosin, and profilin. Both mutations interfered with the polymerization of the actin, and with its interaction with myosin. The C374S mutation had the most pronounced effect; it reduced the polymerizability of the actin, abolished its binding to profilin, and filaments containing this mutation moved at reduced rates in the in vitro 'motility assay'. The ATPase activity measured in solutions containing myosin subfragment 1 was similar for both the mutant and wild-type actins.

Actin mutant; Heterologous expression; Yeast; Polymerization; Actomyosin interaction; Profilin interaction

## 1. INTRODUCTION

The relationship between the crystal structure of actin [1] and its functions in various contexts is currently subject to intensive investigation (for reviews see [2–6]). Site-specific mutagenesis, coupled with analysis of the properties of the mutagenized actin alone, together with myosin, and together with other actin-binding proteins, is used in searching for amino acid residues or structural domains in actin that are crucial to the transduction of energy by the system [7–13]. Such in vitro studies are complemented by studies of the effect of the specific mutagenesis in vivo using different model systems [14–18].

*Saccharomyces cerevisiae* containing actin mutated at position 32 has a temperature-sensitive phenotype and an aberrant actin organization [19], and limited proteolysis of actin in this region interferes with the translocation of actin filaments in the in vitro 'motility assay' [20]. This position is close to residues 39–46, which have been suggested to form a major interaction surface for the binding of DNase I [2], and to participate in monomer:monomer associations in the actin filament [21]. This DNase I binding loop is located in subdomain II of actin, while Cys-374 is found in the opposite end of the monomer where it constitutes part of the profilin binding site [22,23]. The adjacent C-terminal residue, Phe-375, has been suggested to participate in monomer

interactions in the actin filament [21]. In addition, a mutation at position 372 in the actin of the indirect flight muscle of *Drosophila* impairs its flight capacity [14], and there is evidence that residues adjacent to C374 somehow participate in the association with myosin [24–26]. Therefore it is of interest to mutagenize these regions of the actin to locate the sites involved in the actin–actin interaction and in actomyosin-based energy transduction.

We have described the expression and isolation of mutant  $\beta$ -actins from the yeast *S. cerevisiae* [9–11]. Here two such mutations, one with proline at position 38 replaced by alanine (P38A), and a second replacing cysteine at position 374 with a serine residue (C374S), were studied using an arsenal of assays probing for the actin:actin interaction, actomyosin complex formation and force generation. In addition, the binding of the mutant actins to DNase I, and profilin were investigated.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and the DNase I affinity column

Adenosine triphosphate (ATP), dithiothreitol (DTT) and bovine pancreatic deoxyribonuclease I (DNase I; EC 3.1.21.1) were from Boehringer, Mannheim. Hydroxyapatite (Hypatite C, lot No. 6654) was from Clarkson Chemical Co., Williamsport, PA, USA. Cyanogen bromide-activated Sepharose 4B (Pharmacia-LKB, Sweden) was coupled with 500 mg DNase I according to the instructions provided by the manufacturer but excluding the recommended washing of the resin at low pH. The volume of the column was 80 ml.

### 2.2. Production of recombinant actin

Manipulations of DNA followed standard protocols [27] and the

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amino acid replacements were introduced by oligodeoxynucleotide-directed mutagenesis. The expression and preparation of the wild-type and mutant  $\beta$ -actins were performed using the *S. cerevisiae* strain, K923 (HML $\alpha$ , mat::LEU2<sup>+</sup>, hmr::TRP1<sup>+</sup>, ura3, ade2, sir3ts, MAT $\alpha$  at 23°C, MAT $\alpha$  at 34°C) as previously reported [9–11,28].

### 2.3. Protein preparations

Myosin subfragment-1 (S1) was prepared by chymotryptic cleavage of rabbit skeletal muscle myosin [29] and stored at -20°C in 5 mM imidazole, pH 7.0, 15 mM NaCl, 0.1 mM phenylmethylsulfonylfluoride and 50% glycerol.

Human profilin was expressed in *S. cerevisiae* and isolated as described in [30] except that a 100–360 mM KCl gradient was used to elute the material from the phosphocellulose P11 column. This led to the separation of two species of the recombinant profilin; one with a blocked and one with an unblocked N-terminus [31]. In the interaction studies with actin presented here, only N-terminally blocked profilin was used.

Protein concentrations were determined by the method of Bradford [32] or spectrophotometrically at 290 nm for actin using an extinction coefficient of 0.63, and at 280 nm for S1 and profilin using extinction coefficients of 0.75, and 1.1, respectively.

### 2.4. Electrophoresis

This was carried out in SDS-containing 15% polyacrylamide gels (SDS-PAGE) according to Matsudaira and Burgess [33]; isoelectric focusing was done in flat-bed gels with a pH range of 5.0–7.0 as described previously [10].

### 2.5. Assays

The DNase I inhibition was assayed according to Blikstad et al. [34] using a Shimadzu UV-2101PC.

For determination of  $A_{cc}$  (the critical concentration of actin), actin samples (0.3 mg/ml in 300  $\mu$ l G-buffer) were incubated with salt (2 mM MgCl<sub>2</sub> or 100 mM KCl) for 2 h at 25°C after which the DNase I inhibition assay was used to determine the concentration of the unpolymerized actin ( $A_{cc}$ ), as described before [9].

The viscosity analysis was performed at 25°C using Cannon-Manning viscometers and 0.7 ml samples containing 0.5 mg/ml of actin.

The actin-activated S1 ATPase activity was determined at 25°C in G-buffer containing 2 mM MgCl<sub>2</sub>, 25  $\mu$ g/ml S1, and varying concentrations of filamentous actin in a total volume of 0.5 ml to which 1  $\mu$ Ci of [<sup>32</sup>P]ATP was added. Aliquots of 100  $\mu$ l were withdrawn at 3 min intervals and inorganic phosphate was determined by the method of Seals et al. [35]. The data obtained were aligned to Michaelis-Menten kinetics using non-linear regression analysis (Enzfitter, Biosoft).

The motility assay was performed as described by Kron and Spudich [37]. Rabbit skeletal muscle myosin (100–300  $\mu$ g/ml) in 25 mM imidazole, pH 7.0, 25 mM KCl, 5 mM MgCl<sub>2</sub>, 100 mM DTT (assay buffer) was coated on a glass coverslip, and subsequently exposed to wild-type and mutant actin filaments labeled with rhodamine phalloidin (Molecular Probes, OR, USA). After addition of assay buffer containing 1 mM ATP, filament movement was followed using a Zeiss photomicroscope III equipped for epifluorescence. Video recordings were made with a CCD camera (C2400), an image intensifier and a processor (Argus-10) from Hamamatsu Photonics, and a super-VHS videorecorder from Panasonic. For determination of the velocities of the moving filaments, the video signal was time-base corrected with a 780 Synchronizer-TBC (BLT). The velocity of an individual filament was determined from the video recordings by a minimum of 5 measurements of the distance translocated per unit time. To avoid the influence of 'staling' during the translocation, video sequences (recorded at 30 frames/s) were selected after 'frame-by-frame' analysis, and, although the velocity determinations were made over relatively short periods of time (usually 2–8 s), only those filaments that performed a longer continuous movement were chosen for this purpose. All three actins were analyzed with the same myosin preparation and during the same experimental session to avoid changes in velocity due to alterations in the quality of the myosin or to changes in the condi-

tions. The velocity of wild-type actin from time to time was highly reproducible; e.g. 2.7  $\mu$ s (2.703) S.D. = 0.55,  $n$  = 11 obtained in the current study is to be compared with 2.7  $\mu$ s (2.724) S.D. = 0.47,  $n$  = 34 obtained 12 months earlier (the  $P$  value is 0.91).

### 2.6. Binding studies

The profilin:actin interaction was studied by adding yeast-expressed human profilin (see above) in increasing amounts to samples of G-actin in G-buffer. Polymerization was then initiated by the addition of MgCl<sub>2</sub> to 2 mM. After incubation for 2 h at room temperature the extent of polymerization was determined by DNase I inhibition measurements directly on the samples as well as on supernatants obtained by centrifugation at room temperature in a Beckman airfuge for 20 min at 30 psi. The  $K_{diss}$  values were estimated as described in [30,38].

## 3. RESULTS

### 3.1. DNase I inhibition and polymerizing activity

The two actin mutants, P38A and C374S, were isolated from yeast homogenates by affinity chromatography on DNase I-Sepharose. This, and the observed specific DNase I inhibiting activities of the mutants (Table I), shows that the ability of the mutant actins to bind DNase I had not changed significantly compared to wild-type actin. The mutations also did not change the electrophoretic mobility of the actin in polyacrylamide gels, or change its behaviour during isoelectrofocusing (Fig. 1).

However, both the P38A and the C374S mutations interfered with the polymerization of the actin, and the two actins differed in their response to MgCl<sub>2</sub> and KCl as shown in Table I and Fig. 2. The critical concentration for polymerization in the presence of 2 mM MgCl<sub>2</sub> ( $A_{cc,Mg}$ ), as measured with the DNase I inhibition assay, was 0.03 mg/ml for wild-type actin, 0.04 mg/ml for P38A, and 0.16 mg/ml for C374S actin. The corresponding values measured under KCl conditions were 0.16, 0.25, and 0.26 mg/ml, respectively. Thus, whereas the  $A_{cc,Mg}$  for P38A actin remained close to that of wild-type actin, the  $A_{cc,Mg}$  for C374S was increased more than 5 times. This demonstrates that the C-terminus of actin

Table I

Critical concentration of polymerization ( $A_{cc}$ ) for wild-type and mutant actins as determined with the DNase I inhibition assay

| Actin                     | $A_{cc}$                    |                                  |                | Specific activity              |                   |
|---------------------------|-----------------------------|----------------------------------|----------------|--------------------------------|-------------------|
|                           | Mg <sup>2+</sup><br>(mg/ml) | Mg <sup>2+</sup> +E <sup>a</sup> | K <sup>+</sup> | K <sup>+</sup> +E <sup>a</sup> | U/mg <sup>b</sup> |
| Wild-type                 | 0.029                       | 0.025                            | 0.159          | 0.062                          | 41,749            |
| (S.D./ $n$ ) <sup>c</sup> | 0.006/6                     | 0.005/7                          | 0.010/7        | 0.005/6                        | 2,058/5           |
| P38A                      | 0.037                       | 0.039                            | 0.245          | 0.080                          | 39,128            |
| (S.D./ $n$ ) <sup>c</sup> | 0.012/13                    | 0.010/13                         | 0.016/11       | 0.019/7                        | 2,345/8           |
| C374S                     | 0.159                       | 0.157                            | 0.263          | 0.197                          | 38,803            |
| (S.D./ $n$ ) <sup>c</sup> | 0.008/9                     | 0.012/9                          | 0.017/7        | 0.033/10                       | 5,350/8           |

<sup>a</sup> [Ethylenebis(oxonitrilo)]tetraacetic acid (EGTA).

<sup>b</sup> DNase I inhibition units per mg of actin as defined in [34].

<sup>c</sup> S.D. = standard deviation,  $n$  = number of observations.

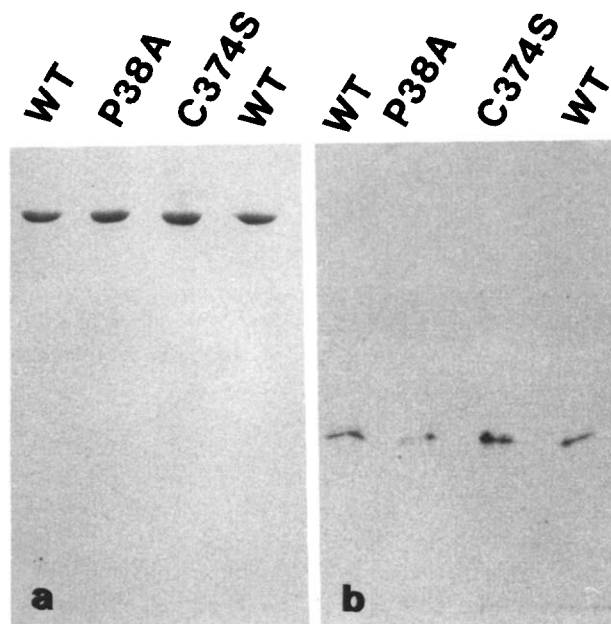


Fig. 1. The homogeneity of the yeast-expressed  $\beta$ -actins as seen by polyacrylamide gel-electrophoresis (a), and isoelectrofocusing (b). Lanes displaying wild-type actin are denoted WT, and those with mutant actins, P38A and C374S.

plays an important role in the polymerization process. The increased  $A_{cc}$  values observed for the mutant actins in KCl conditions implies that both subdomains I and II of actin [1] participate in monomer:monomer interactions in the actin filament.

Fig. 2 shows the results of the viscosity analysis of the polymerization of the two mutant actins as compared to wild-type actin. In  $MgCl_2$  all three actins reached half maximal viscosity 2–4 min after the initiation of the reaction. The only significant difference between the three actins was that the C374S actin reached a lower steady-state level of viscosity. These assays were performed with samples containing 0.5 mg/ml actin. Since the  $A_{cc,Mg}$  value for the wild-type actin obtained with the DNase I inhibition assay was 0.03 mg/ml, the relative viscosity at steady state, 1.24, corresponds to a polymerization of 94% of the actin. Assuming a linear relationship between the concentration of filamentous actin and the relative viscosity, and that the overall hydrodynamic properties of the wild-type and mutant filaments are similar, the  $A_{cc}$ 's can be calculated. The observed relative viscosities at steady state for the P38A and C374S mutant actins (1.26 and 1.20, respectively) give  $A_{cc,Mg}$  values (0.02 mg/ml and 0.13 mg/ml) which are in reasonable agreement with those obtained by the DNase I inhibition assay (0.04 and 0.16 mg/ml, respectively), suggesting that P38A actin polymerized like wild-type actin whereas the polymerization of C374S actin was reduced by 25–30%. However, ultracentrifugation of the samples before determination of DNase I inhibition (see profilin interaction studies below) suggested that

the DNase I inhibition activity determined for C374S actin, to a large extent (approximately 70%), was caused by actin in a pelletable form. The reason for this discrepancy is unclear. It is possible that it is due to rapid depolymerization of a fraction of the actin during the DNase I inhibition assay itself. Another possibility is that the mutant actin filaments themselves bind DNase I.

The filament formation is less efficient when induced by  $K^+$  ions [9,10,38], therefore, these conditions probe more subtle influences by the mutations on the polymerization process than those which include  $Mg^{2+}$ . Under KCl conditions the kinetics of filament formation with both mutant actins differed from that of wild-type actin. The P38A actin reached half maximal viscosity only after 47 min as compared to 25 min for wild-type actin. At the protein concentration used the C374S actin did not lead to any detectable filament formation. However, the rapid increase in viscosity seen on addition of  $MgCl_2$  demonstrated that the C374S actin was still capable of forming filaments (Fig. 2). Thus, qualitatively the viscosity analysis was in agreement with the results obtained with the DNase I inhibition assay. With the wild-type actin also, the apparent extent of polymerization was the same when either the DNase I inhibition assay or viscosity analysis was used (compare  $A_{cc,K} = 0.16$  mg/ml with  $\eta_{rel} = 1.18 \Rightarrow A_{cc} = 0.17$  mg/ml), but with the mutant actins this was not the case. With P38A actin

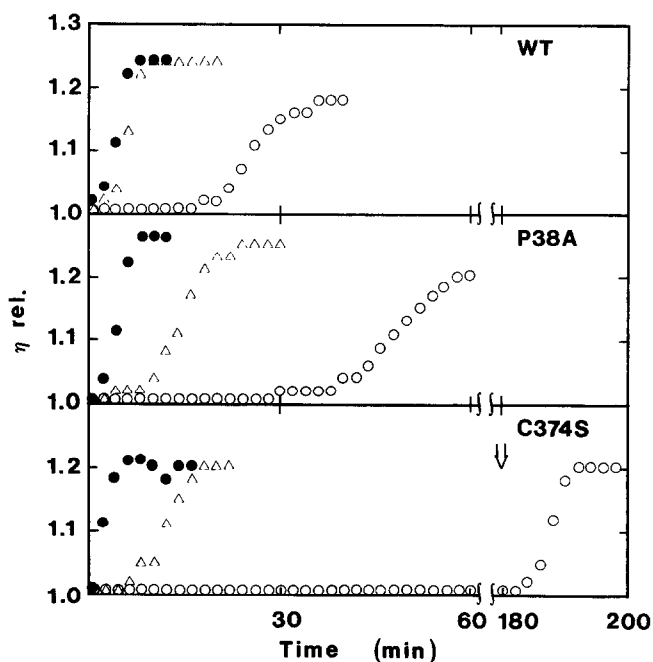


Fig. 2. The polymerizability of the wild-type and mutant actins analyzed by viscosity measurements. The relative viscosity ( $\eta$ ) of samples containing the different actins (labeled as in Fig. 1) was determined as a function of time after addition of salt. (○) 100 mM KCl; (●) 2 mM  $MgCl_2$ ; (△) 100 mM KCl + 0.3 mM EGTA. The arrow in the C374S actin (bottom) panel indicates addition of  $MgCl_2$  to 2 mM to the KCl sample.

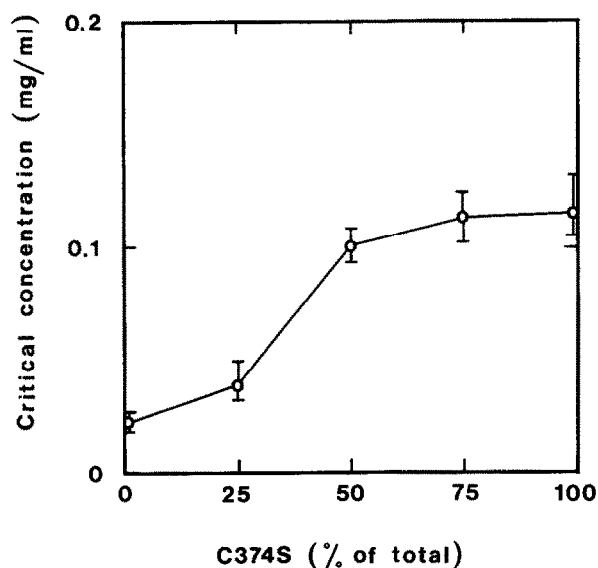


Fig. 3. Co-polymerization of wild-type and C374S mutant actin. The  $A_{cc,Mg}$  was determined by the DNase I inhibition assay for the different combinations of the two actins. The concentration of actin was 0.3 mg/ml. Error bars indicate the variation in two or three determinations.

the viscosity analysis suggested a more efficient polymerization than was indicated by the DNase I inhibition assay,  $\eta_{rel} = 1.21$  instead of 1.14 as derived from the  $A_{cc,K}$  value. This suggests that the P38A mutation changed the DNase I binding characteristics of the actin, possibly by also making the DNase I binding site on actin available in the filament. With C374S actin, on the other hand, no polymerization was observed with the viscosity assay, indicating that stable filaments were not formed in KCl conditions. Hence, either these filaments were extremely fragile, or of oligomer length only.

The effect of the C374S mutation on actin polymerization was studied further by measuring the  $A_{cc,Mg}$  in mixtures of mutant and wild-type actin. As shown in Fig. 3 the  $A_{cc,Mg}$  was nearly doubled when the mutant protein amounted to 25% of the total actin. When the mutant and wild-type actins were present in equimolar amounts, the  $A_{cc,Mg}$  value was already 5-times that obtained with wild-type actin alone, or equal to the  $A_{cc,Mg}$  of isolated C374S actin. This shows that the two actin species formed co-polymers, and that monomer interactions other than those involving residue 374 can not balance the deleterious effect of the C374S mutation. Thus, the C-terminus of actin plays a central role in filament formation.

As observed previously, EGTA influences KCl-induced polymerization such that it becomes similar to that seen with  $MgCl_2$ . This is particularly prominent with non-muscle actins, and in the case of  $\beta$ -actin it has been suggested that an additional  $Ca^{2+}$ -binding site is present on the molecule [10,36]. Therefore the polymerization behaviour of the recombinant actins was also analyzed in conditions where EGTA had been added to a concentration of 0.3 mM. This results in a free  $[Ca^{2+}]$  of  $10^{-7}$  M, which is about two orders of magnitude above the  $K_{diss}$  for the high-affinity  $Ca^{2+}$  binding site on actin. Previously, we have reported that this treatment has no influence on  $Mg^{2+}$ -induced polymerization, while it enhances the polymerizability in KCl conditions [9,10]. This was also the case in the current study. With both wild-type and mutant actins, EGTA reduced the  $A_{cc,K}$  values towards those obtained in the presence of  $Mg^{2+}$  ions (Table I). The C374S mutant actin did not respond to the removal of  $Ca^{2+}$  to the same extent as the P38A and wild-type actin. With the C374S actin the reduction in  $A_{cc,K}$  was 29% as compared to 62 and 68% for wild-type and P38A actin, respectively. The reason for this is unclear but is consonant with the more

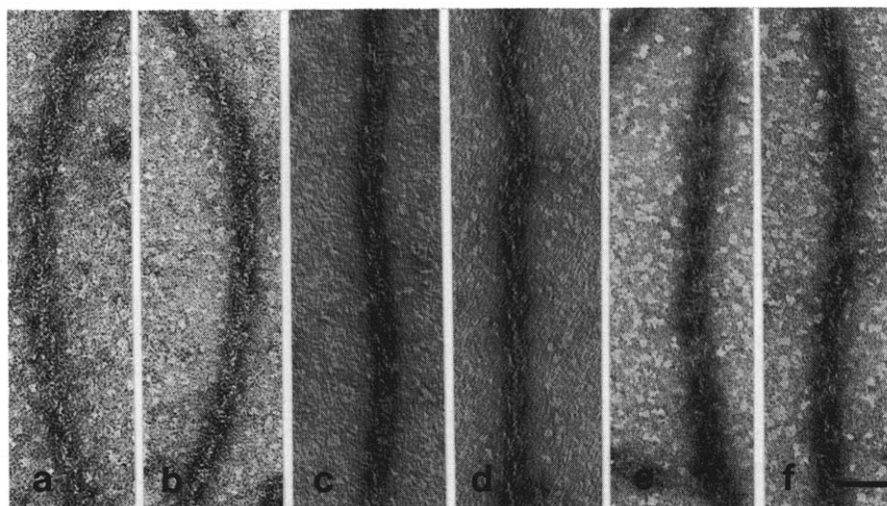


Fig. 4. Decoration of wild-type and mutant actin filaments with myosin subfragment 1. (a and b) Wild-type actin; (c and d) P38A actin; and (e and f) C374S actin. The specimens were stained with 0.25% uranyl acetate. Bar = 50 nm.

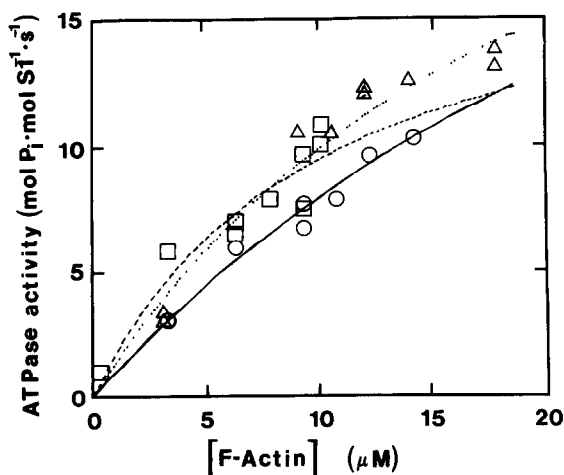


Fig. 5. ActoS1-ATPase activities with the wild-type and mutant actins. The solid line (○) indicates the result with wild-type actin, and that of P38A and C374S actin is shown by a dotted (△) and a dashed line (□), respectively. The concentration of filamentous actin was calculated on the basis of the  $A_{cc}$  for the different actins, and the plot was obtained by a best-fit alignment to Michaelis-Menten kinetics using the Biosoft Enzfitter program.

strongly disturbed polymerization behaviour for the C374S mutant actin.

### 3.2. Myosin interaction

Decoration of P38A and C374S mutant actin filaments with myosin subfragment 1 (S1) resulted in the typical acto-S1 arrowhead pattern (Fig. 4). Thus, neither mutation seemed to influence the formation of rigor links with myosin. As shown in Fig. 5, the ATPase activity obtained with S1 and the mutant actin filaments in solution were close to that obtained with wild-type actin. This suggests that residues P38 and C374 are of minor importance to the step in the actomyosin interaction that results in product release from the myosin.

However, when probing for the ability of the actin filaments to translocate on immobilized myosin in the *in vitro* motility assay, both the P38A and C374S mutations showed a decreased activity. The P38A actin filaments moved with a mean velocity of  $2.2 \mu\text{m/s}$  (S.D. = 0.5;  $n = 22$ ), and with C374S mutant actin the corresponding value was  $1.8 \mu\text{m/s}$  (S.D. = 0.4;  $n = 19$ ). This is compared to  $2.7 \mu\text{m/s}$  (S.D. = 0.6;  $n = 11$ ) for wild-type actin. The 33% reduction in the rate of translocation of the C374S mutant actin is highly significant with  $P = 0.0002$  in a two sample *t*-test, while the effect of the P38A mutation was less dramatic (18% reduced velocity;  $P = 0.03$ ). Thus, the two mutations appear to discriminate between two steps in the actomyosin interaction; one expressed as binding of myosin subfragment S1 and ATPase activity in solution, and the other observed as translocations of the filaments in the *in vitro* motility assay.

### 3.3. Profilin interaction

Profilin inhibits the polymerization of wild-type  $\beta$ -actin in the presence of 2 mM  $\text{MgCl}_2$ , as shown in Fig. 6. The inhibitory effect is seen already at a profilin:actin molar ratio of 0.2. At a molar ratio of 1, there was a 6-fold increase in the concentration of unpolymerized actin in steady state with filamentous actin (from 0.03 mg/ml to 0.18 mg/ml). The P38A actin behaved similarly, reaching plateau values of unpolymerized actin at molar ratios of profilin:actin of about 1.5:1.

Supernatants, obtained by high-speed centrifugation of samples containing actin polymerized in the presence of increasing amounts of profilin, were also analysed using the DNase I inhibition assay as shown in Fig. 6b. This procedure which avoids an over-estimation of unpolymerized actin because of filament ends interacting with DNase I, had a prominent effect on the DNase I inhibition activity determined for the C374S actin. As already mentioned, results obtained after ultracentrifugation suggested a lower  $A_{cc}$  value for this mutant actin (0.039 mg/ml), while this procedure only marginally influenced the  $A_{cc}$  values determined for the wild-type and P38A actins ( $A_{cc} = 0.017 \text{ mg/ml}$  in both cases). In the presence of profilin, however, also the wild-type and P38A actins showed a reduced DNase I inhibition activity after centrifugation, suggesting the presence of pelletable and DNase I-interacting complexes of actin under these conditions. From the data in Fig. 6b, the dissociation coefficient ( $K_{diss}$ ) for the profilin:actin interaction was determined at  $0.3 \mu\text{M}$  (S.D. = 0.2) and  $0.9 \mu\text{M}$  (S.D. = 0.9) for the profilin:P38A actin and profilin:wild-type actin, respectively ( $P = 0.0086$ ). The somewhat tighter interaction of profilin with P38A actin, as compared to that with wild-type actin, thus seems to indicate that changes in subdomain II may influence the profilin:actin interaction, although the

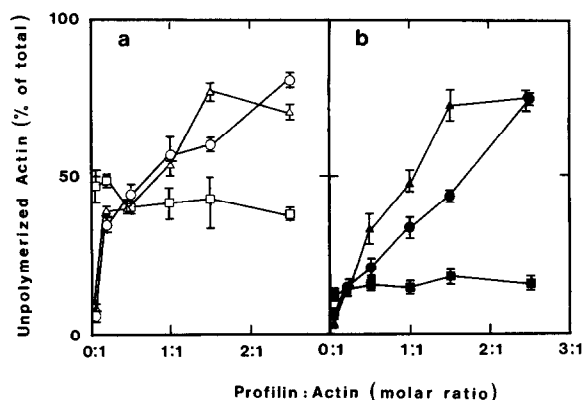


Fig. 6. Formation of profilin:actin complexes in the presence of 2 mM  $\text{MgCl}_2$ . The samples containing profilin and actin (0.3 mg/ml) in the molar ratios indicated on the abscissa were analyzed for the presence of unpolymerized actin by the DNase I inhibition assay before (a) and after (b) ultracentrifugation. Mean values of 2–4 determinations obtained with wild-type actin (circles), and with P38A and C374S actin (triangles and squares, respectively).

major profilin binding site on actin is located in subdomains I and III (C.E. Schutt et al., submitted).

When profilin was added to C374S mutant actin, on the other hand, there was no inhibition of the polymerization of the actin. The level of polymerization was in agreement with the  $A_{\infty}$  value determined for this actin, and it remained constant regardless of the presence of increasing concentrations of profilin (Fig. 6). This result therefore confirms earlier observations that manipulations with the C-terminus of actin interfere with the profilin:actin interaction, and it emphasizes the central role of residue C374 in forming the profilin-binding site on actin. The same result was obtained when analyzing the high-speed supernatants (Fig. 6b) except for the observation that a major part of the DNase I inhibiting activity was pelletable as described above.

#### 4. DISCUSSION

The P38A mutation introduced into  $\beta$ -actin is located close to the surface loop contributing to the major DNase I binding site (residues 38–45) on subdomain II [2]. However, as shown here, the P38A mutation did not seriously influence the ability of the actin to bind to DNase I. This is understandable since this amino acid replacement should merely increase the flexibility of the polypeptide chain in the neighbourhood of P38. Also, the additional DNase I-binding sites present on subdomains II and IV of actin [2] might guide DNase I into an interaction of similar tightness as that seen with wild-type actin.

Results of various types of chemical experiments have suggested that the DNase I-binding loop is involved in inter-monomer binding in the actin filament. This is supported by the observation that polymerization of P38A actin is slightly aberrant, as seen by a prolonged lag-phase and an increased  $A_{\infty,K}$  value. This is consonant with the filament model of Holmes and co-workers [21], as well as with the ribbon structure of actin conjectured to be an extended and untwisted form of helical actin [39]. Thus, it is not possible to discriminate between these two filament models on the basis of the current data, even though the pattern of interactions in the two models are completely different (C.E. Schutt et al., submitted).

The strong interference of  $Mg^{2+}$ -induced polymerization by the C374S mutation supports the observation that the actin C-terminus plays an important role in stabilizing monomer:monomer interactions in the filament [21,40]. This is particularly evident from the copolymerization experiment with wild-type actin described in Fig. 3. Our data therefore points towards subdomain I, and in particular the C-terminus, as forming a major inter-monomer binding site in the actin filament.

The P38A mutation led to a small but significant decrease in the rate of actin filament translocation in the

in vitro motility assay, while decoration of the mutant actin with myosin S1, and the ATPase activity of acto-S1, was not significantly changed. The C374S actin behaved similarly, but the movement of these mutant filaments was more strongly suppressed. The effect was of the same magnitude as that seen with mutations introduced at the actin N-terminus where the aspartic acid residues, D3 and D4, were deleted or replaced with alanines [9,11]. However, these latter mutations also altered the decoration of the actin with myosin S1, and decreased the activity of the acto-S1 ATPase measured in vitro, demonstrating a more severe disturbance of the acto-myosin interaction in those cases.

Thus, mutations in actin can impair the chemo-mechanical transduction of the system, even though the acto-myosin rigor complex can form, and the ATPase activity of mixtures of actin and myosin in solution is retained. In the standard cross-bridge theory, the relative movement of actin and myosin filaments is directly coupled to the myosin ATPase activity, and the velocity of the filaments should be independent of the number of 'motor' molecules acting on them (for reviews see [43,44]). The mutations introduced into actin in the present experiments, however, appear to have interfered with the coupling of the chemical reactions to mechanical events. One interpretation of this is that the processes of acto-myosin-induced ATP hydrolysis and force generation engage different actin-myosin contacts, and that the first process can proceed relatively independent of the second. Alternatively these data may be interpreted in view of the new model of energy transduction in the acto-myosin system that was recently proposed [45]. This model retains the element of tight coupling between myosin-binding and the myosin ATPase activity, while placing force production in the actin molecule. The energy from the hydrolysis of ATP on myosin is used merely to initiate a transformation of the actin filament into a metastable, stretched state (ribbon actin). Movements are generated when myosin-linked actin is returning to the ground state (helical actin). Interpreted in terms of the new model, this would mean that the initial myosin-dependent activation step is unaffected, but that the steps involving length changes in actin have been altered by the mutation. Results consonant with these ideas have also been obtained by others [20,46–49].

Under KCl conditions (50 mM KCl; no  $Mg^{2+}$  added) profilin is an effective inhibitor of actin polymerization. It forms a stable 1:1 complex with  $\beta$ -actin from the same source ( $K_{diss} < 10^{-8}$  M), and if filamentous  $\beta$ -actin is incubated with equimolar amounts of profilin in the absence of  $Mg^{2+}$ , the filaments are rapidly depolymerized, while in the presence of 1 mM  $Mg^{2+}$  plus 50 mM KCl, the  $K_{diss}$  is increased more than 10-fold [36]. If the actin and profilin are from heterologous sources the complex formed is less stable, as indicated by a higher  $K_{diss}$  value ( $>10^{-6}$  M) [36,50]. The  $K_{diss}$  value for the

profilin:wild-type actin complex determined in this study ( $0.9 \times 10^{-6}$  M) is in agreement with that determined by others for a profilin:*non-muscle* actin interaction [51], and it is in the same range as that determined for the interaction between actin and thymosin- $\beta_4$ , which is also known to be an actin monomer binding protein [50]. The reason for the slightly reduced  $K_{\text{diss}}$  value observed for the profilin interaction with P38A actin is unknown.

Removal of the C-terminal Phe-375 [52], or chemical modification of the penultimate cysteine residue [22,23], makes the actin resistant to profilin. This strongly suggests that profilin interacts with the C-terminus of actin, and that profilin thus should be a sensitive probe for structural changes in the C-terminal part of the actin molecule. The results obtained with the C374S mutant actin confirms that the structure of the actin C-terminus is of decisive importance for the formation of the profilin:actin complex. It is remarkable that the replacement of a cysteine SH-group with a serine OH-group can have such a dramatic effect on the profilin:actin interaction. It is possible that the C374S mutation causes the disruption of a larger structure element normally contributing to the formation of the profilin binding site on actin. It is noteworthy that the stabilization of profilin:actin crystals seemed to be especially sensitive to mercurials and other alkylating agents, and their use had to be avoided in obtaining the heavy-atom derivatives needed for phasing the structure factors [40]. Several lines of evidence suggest that the C-terminal region is part of a sensitive mechanism influencing the behaviour of the actin molecule.

Recent evidence from genetic studies in yeast confirms that the C-terminus of the actin molecule is physiologically important [17]. Deleting one, two or three residues from the C-terminal (-KCF<sub>COOH</sub>) sequence causes increasing disturbance of the yeast phenotype, with lethality as the ultimate consequence when all three are removed. The same report showed that mutations at residues 373 or 374 (K373M and C374A), introduced separately or together, had no effect on cell viability. In all three cases, the yeast cells appeared to exhibit a wild-type phenotype. This reinforces the concept of a physiologically important and sensitive structure involving the C-terminal amino acid sequence -KCF<sub>COOH</sub>.

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