Evidence for two interaction regions for phosphatidylinositol(4,5)-bisphosphate on mammalian profilin I

Petra Skare, Roger Karlsson*

Department of Cell Biology, Wenner Gren Institute, Stockholm University, S-106 91 Stockholm, Sweden

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Abstract The binding of phosphatidylinositol(4,5)-bisphosphate ($PI(4,5)P_2$) to profilin at a region distinct from the actin interaction surface is demonstrated by experiments with covalently cross-linked profilin:β-actin. The result is in agreement with observations made with several mutant profilins and provides strong evidence for two regions on mammalian profilin mediating electrostatic interaction with phosphatidylinositol lipids; one close to the binding site for poly(L-proline), and one partially overlapping with the actin-binding surface. Congruent with this, two plant profilins, which have a reduced number of positive amino acids in one of these regions, displayed a dramatically lower binding to $PI(4,5)P_2$ compared to human profilin I. **© 2002 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

Key words: Profilin:actin; Phosphatidylinositol(4,5)-bisphosphate; Actin dynamics; Profilin mutants

1. Introduction

Profilin is a principal regulatory component of actin organization [1,2]. In complex with non-muscle actin (β and γ isoforms), profilin efficiently interferes with filament formation unless proper nucleation sites like free filament (+)-ends are available [3–5]. Recent studies in vitro [6–8] have provided evidence for a model [9] where interaction of profilin:β/γ-actin with filament (+)-ends leads to dissociation of profilin and final incorporation of the actin into the filament. If profilin does not dissociate readily from actin as with a mutant that binds actin with a lowered K_d compared to the wild-type [6], or does not dissociate at all as with a covalently coupled profilin:actin complex [8], little or no productive incorporation of actin into the filament occurs. Instead the complex is released leaving the filament end free to participate in new interactions. In the cell, the cross-linked complex causes complete derangement of the microfilament system at the advancing edge [10], supporting the view that profilin:actin is the immediate precursor to actin polymerization [2,11] and suggesting additional mechanisms involved in making the actin in profilin:actin available for filament growth in vivo.

Profilin has several interaction partners in addition to actin [12]. Many of these, like members of the Ena/Vasp and formin families of proteins, bind profilin at the poly(L-proline)-binding site, a binding surface commonly employed for isolation of profilin on polyproline-coupled matrices [13]. The binding sites for other interaction partners like Arp2 in the Arp2/3 complex and the p85 regulatory subunit of PI3 kinase have not yet been defined. The property of profilin to bind $PI(4,5)P_2$ in vitro has led to an extensive interest since this interaction causes profilin:actin to dissociate [14] and thereby could connect the control of actin polymerization with processes involved in signal transduction, e.g. [12,15]. Despite this, the binding surface for $PI(4,5)P_2$ on profilin has not been clearly identified, nor has the role of the profilin–$PI(4,5)P_2$ interaction been demonstrated in vivo. In *Saccharomyces cerevisiae*, however, conditions which cause depletion of phosphatidylinositols lead to redistribution of profilin from the plasma membrane to the cytoplasm as if the interactions with $PI(4,5)P_2$, and perhaps also $PI(3,4)P_2$ and $PI(3,4,5)P_3$ [16], are important for its location at the cell periphery [17]. In addition, several reports have shown that $PI(4,5)P_2$ is closely connected to microfilament dynamics in vivo, e.g. [18–20].

This study shows that a ternary complex can form between profilin:actin and $PI(4,5)P_2$, extending earlier observations of the interaction between this lipid and the profilin:actin complex [21]. The effect of several point mutations in profilin on the interaction with $PI(4,5)P_2$ is also described.

2. Materials and methods

2.1. Mutagenesis and protein purification

Mutagenesis of the human profilin I gene, cloning and expression in *S. cerevisiae*, and subsequent isolation of the recombinant protein were performed as described previously [6,22,23]. *Arabidopsis thaliana* profilins 1 and 3 were kindly provided by Dr. H. Christensen, National University of Singapore. For interaction studies with $PI(4,5)P_2$ micelles, the proteins were dialyzed into buffer A (10 mM KPO_4 pH 7.6, 80 mM NaCl, 0.1 mM $CaCl_2$, 0.12 mM EGTA and 1 mM DTT). Concentrations were determined spectrophotometrically at 280 nm [6]. Profilin:actin was purified according to [13,24], chromatographed in G buffer (10 mM KPO_4 pH 7.6, 0.1 mM CaCl, 0.12 mM EGTA, 10 μM EDTA, 0.5 mM ATP and 1 mM DTT) on Sephacryl S300 (Amersham Biosciences) and stored as an ammonium sulfate precipitate at 4°C.

2.2. Preparation of covalently cross-linked profilin:actin and co-chromatography with $PI(4,5)P_2$ micelles for interaction studies with profilin:actin

For covalent coupling of profilin to actin, profilin:actin was dissociated, and the proteins isolated [13] followed by covalent coupling by EDC/NHS as described by [8]. Phosphatidylinositol(4,5)-bisphosphate was either purchased from Boehringer Mannheim, or isolated from calf brain as in [23]. The profilin:actin, non-cross-linked and cross-linked, was transferred into G buffer and either loaded directly on a

*Corresponding author. Fax: (46)-8-159837.
E-mail address: roger.karlsson@cellbio.su.se (R. Karlsson).

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FPLC Superdex 200 gel filtration column (Amersham Biosciences) or, prior to chromatography, was incubated for 15 min at room temperature with $PI(4,5)P_2$ micelles prepared (see below) from 4 M $PI(4,5)P_2$ in G buffer containing 7.4 kBq ^{14}C-labeled phosphatidylinositol [14]. Fractions (0.6 ml) were collected and measured spectrophotometrically at 232 nm, and every other was analyzed for either protein content by SDS–PAGE or presence of lipid micelles by scintillation analysis.

2.3. Analysis of the $PI(4,5)P_2$–profilin interaction

The binding of $PI(4,5)P_2$ to profilin was determined with a filter assay modified from Haarer et al. [25]. Micelles were formed by sonication of 0.3 mM $PI(4,5)P_2$ in buffer A for 10 min at 80°C. Different amounts of freshly prepared micelles resulting in the lipid to protein ratios indicated were incubated with 0.5 nmol of profilin in a total volume of 240 μl for 15 min at room temperature or for 30 min on ice; both conditions gave the same final result. After incubation, free and $PI(4,5)P_2$-bound profilin were separated by centrifugation through a Millipore PLTK filter (molecular weight cut off 30 000 Da) prewashed with buffer A. The flow-through (100 μl), containing free profilin was concentrated, analyzed by SDS–PAGE, and the amount of protein in the profilin band was estimated by densitometry. For each ratio of $PI(4,5)P_2$ to profilin analyzed, the densitometry value representing unbound profilin was related to the corresponding value obtained in the absence of $PI(4,5)P_2$. This in turn enabled calculation of the $PI(4,5)P_2$-bound fraction of profilin, i.e. $0.5\times(1-\text{unbound}_{+PIP2}/\text{unbound}_{-PIP2})$ nmol. The values obtained for each sample were plotted against the sample concentration of $PI(4,5)P_2$ and fitted to an exponential curve using Origin 4.1. The slope of the linear part of the curve, mostly in the 0–2.5 nmol concentration range of $PI(4,5)P_2$, obtained for each profilin was related to the slope obtained with wild-type profilin in parallel analyzes. To avoid influences from variations in staining intensities between different gels, samples from each mutant to be analyzed were compared to wild-type samples incubated with the same amounts of $PI(4,5)P_2$ and analyzed on the same gel (Fig. 2A).

3. Results and discussion

3.1. Cross-linked profilin:actin forms a stable complex with $PI(4,5)P_2$ micelles

Several studies addressing the location of the $PI(4,5)P_2$-binding site have been performed with a range of profilins from different sources including mammals, plants and protozoa. Without clearly identifying the most likely interaction surface, these have essentially recognized two possible regions of the molecule as involved, one overlapping with the actin site and one in the nearness of the poly(L-proline) site. This led us to the hypothesis that PIP_2-binding to profilin takes place via two spatially separated interactions rather than

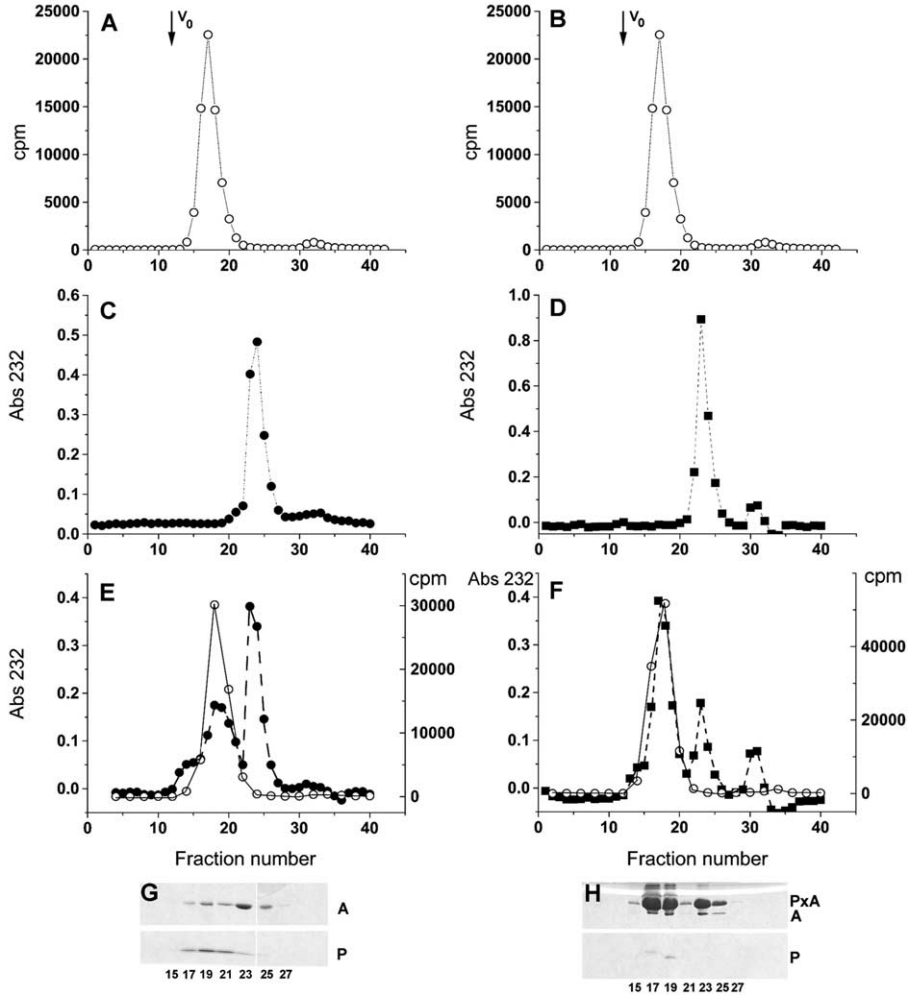

Fig. 1. Gel filtration chromatography of profilin:actin, PxA and $PI(4,5)P_2$ micelles. A and B: $PI(4,5)P_2$ micelles only. C and D: Non-cross-linked profilin:actin and PxA, respectively. E and F: $PI(4,5)P_2$ micelles preincubated with non-cross-linked profilin:actin and PxA, respectively. G and H: SDS–PAGE analysis of the fractions separated in the experiments shown in E and F, respectively. The ^{14}C-labeled $PI(4,5)P_2$ micelles (open circles) were detected by scintillation analysis and the proteins by OD measurement at 232 nm (profilin:actin, filled circles and PxA, filled squares).

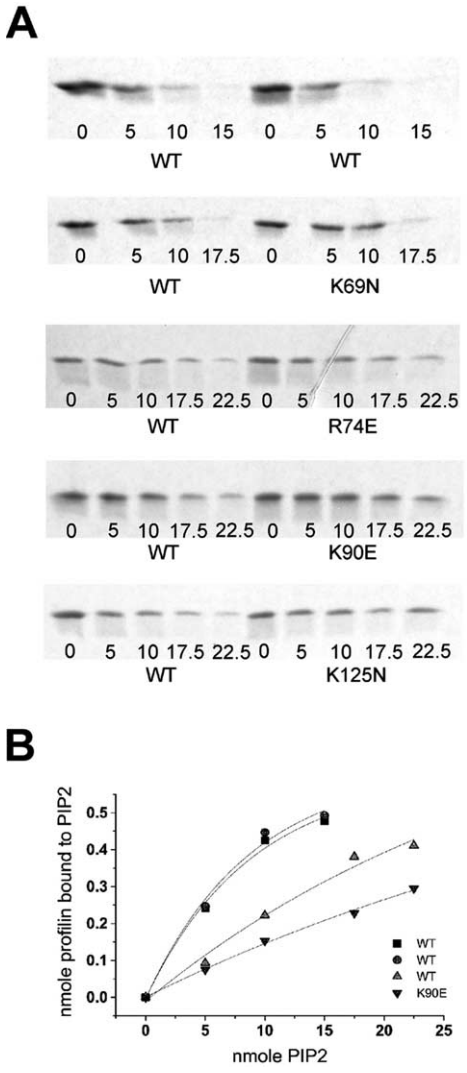

Fig. 2. PI(4,5)P_2-binding properties expressed by wild-type and mutant profilins. A: detection of free profilin by SDS-PAGE after separation of free and PI(4,5)P_2-bound profilin in the filter assay (see text). Typical results with wild-type and four mutant profilins are shown. Numbers at the bottom of the gel lanes refer to the amount of PI(4,5)P_2 in nmol. B: Illustrates how the PI(4,5)P_2-binding properties for the different profilins were compared by plotting the amount of PI(4,5)P_2-bound profilin against sample concentration of PI(4,5)P_2. For clarity, only data derived from densitometry measurements of the top (WT) and middle (K90E) gels displayed in A are shown. The closely similar graphs generated for the two wild-type samples (filled square and filled circle) illustrate that comparison of PI(4,5)P_2-binding capacities using this method is reliable as long as the two sets of samples to be compared are analyzed on the same gel.

through one unique site. To investigate this possibility, we used a non-dissociable profilin:actin complex [8] where residue E82 in profilin is covalently coupled to actin K113 (the one-letter code followed by sequence position is used to denote specific amino acids). Except for the tether, this profilin:actin, called PxA, is closely similar to ordinary profilin:actin [8]. PxA interfered with filament growth in vitro and blocked lamellipodial extension when injected into cultured cells [8,10].

Incubation mixtures of PI(4,5)P_2 micelles and either non-coupled profilin:actin or PxA were analyzed for the presence of macromolecular assemblies by gel filtration chromatography (Fig. 1). When chromatographed separately, the lipid micelles eluted from the column as a homogeneous peak at an earlier position compared to that of profilin:actin or PxA (Fig. 1A–D) in agreement with the larger relative molecular weight of PI(4,5)P_2 micelles in aqueous solution [26].

Preincubation of the PI(4,5)P_2 micelles with profilin:actin in a 1:1 molar ratio (assuming a packing number of 82 lipid molecules in the micelles [26]), dramatically influenced the sample components. Analysis by SDS–PAGE of the fractions after chromatography demonstrated the presence of free actin that eluted from the column at a delayed position compared to the micelles (Fig. 1E and G). In contrast, practically all of the profilin was present in micelle-containing fractions. This shows that the interaction with PI(4,5)P_2 caused profilin:actin to dissociate into free actin and PI(4,5)P_2-bound profilin as originally demonstrated by Lassing and Lindberg [14]. As also noted in [14], a small accumulation of actin (approximately 14% of the total as judged from densitometry of the gel bands) was observed in the major PI(4,5)P_2-containing fraction (Fig. 1E and G). Since the experiment was performed under non-polymerizing conditions and oligomeric actin was not observed in the sample containing profilin:actin alone, we interpret this result to reflect the presence of a ternary complex of profilin:actin and PI(4,5)P_2. The reason why a fraction of the profilin:actin did not dissociate after micelle-binding is unclear.

Evidence for the formation of a ternary profilin:actin–PI(4,5)P_2 complex was obtained by preincubating PI(4,5)P_2 with PxA instead of profilin:actin. This changed the chromatography behavior of a large proportion of the PxA to coelute with the PI(4,5)P_2 micelles instead of eluting in a single peak at the same position as non-cross-linked profilin:actin as was typically seen when PxA was chromatographed alone (Fig. 1C, D and F). Therefore, release of actin from profilin:actin is not required for a stable interaction between PI(4,5)P_2 and profilin, demonstrating the presence of a PI(4,5)P_2-binding region on profilin that is separated from the actin-binding site. In the experiment shown in Fig. 1F and H, a calculated molar ratio of two micelles per PxA was used. According to the gel densitometry, this transferred approximately 60% of total PxA to the lipid fractions, while with a 1:1 molar ratio (not shown) about 40% was transferred. This should be compared to the result shown in Fig. 1E where practically all of the profilin from the ordinary profilin:actin coeluted with PI(4,5)P_2. It appears therefore that the interaction of PxA with the lipid micelles is not as tight as when profilin can separate from actin, suggesting that release of actin unveils surface regions on profilin that contribute to further tightening of the association.

3.2. Analysis of profilin point mutants and two plant profilins for their PI(4,5)P_2 interaction

To analyze specific amino acid residues for their contribution to lipid-binding, different point mutations of profilin [6,23,28] were probed for their micelle-binding capacity by a spin filter assay. Under standardized conditions, free profilin passes through the filter, while free and profilin-bound PI(4,5)P_2 micelles due to their larger size are retained in the non-filtered fraction. Gel analyses of spin-filtered samples of wild-type profilin and the profilin mutants are shown in Fig. 2A. With increasing concentrations of PI(4,5)P_2 in the sample,

Table 1
Summary of PI(4,5)P_2-binding properties expressed by different profilin mutants

Profilin	Change in PIP_2 affinity	PIP_2 affinity relative to wild-type	References
Human			
Wild-type		1.0	this study
A1	binding[a]		[34]
W3N	↑	2.10	this study, [23]
W3F	=		[44]
Y6F	=		[30]
D8A	↑		[30]
K25Q	=		[30]
W31F	=		[44]
K53I	=		[30]
K69N	↓	0.75	this study
R74E	=	1.05	this study
R74L	=		[30]
R88L	↓		[30]
K90E	↓	0.65	this study
P96ΔT97Δ	↑	1.91	this study, [28]
H119D	=		[30]
G121D	=		[30]
K125N	↓	0.71	this study
H133S	=	0.98	this study, [23]
R136D	↓		[40]
S. cerevisiae			
R74E (R72E)[b]	↓		[25]
F83E (R76E)[b]	↓		[25]
R88G (R81G)[b]	=		[25]
F83G (R76G)[b]	=		[25]
F83GR88K (R76GR81K)[b]	=		[25]
Zea mays			
Y6Q (Y6Q)[c]	=		[33]
Y6F (Y6F)[c]	=		[33]
D8A (D8A)[c]	↑		[33]
R88A (K86A)[c]	=		[33]

[a]Interaction observed by cross-linking.
[b]*S. cerevisiae* sequence.
[c]*Z. mays* sequence.

less of the wild-type profilin appeared in the filtrate. For three of the mutants, K69N, K125N and K90E (mutants are named by adding the mutant residue after its position in the sequence), this effect was less prominent indicating reduced lipid-binding capacity. A fourth profilin mutant, R74E, displayed nearly wild-type behavior in this respect.

A quantitative measure of PI(4,5)P_2-binding capacity expressed by the different profilin mutants relative to the wild-type was obtained by densitometry of the profilin band after SDS–PAGE of the filtrate (Fig. 2). This analysis showed that two mutants, R74E, and H133S from a previous study [23], displayed wild-type PI(4,5)P_2-binding properties, Table 1. Of these residues, R74 is part of the actin-binding site while H133 resides in the C-terminal helix and contributes to poly(L-proline)-binding [23,27,28]. Three mutants K69N, K125N and K90E displayed reduced binding with K90E being most severely affected. These positions in profilin are all engaged in actin-binding [29]. Previous studies of two mutations, W3N and the double-deletion P96ΔT97Δ, showed that these changes increased the ability to bind PI(4,5)P_2 [23,28], and Table 1. Both K69 and K90 expose their charged side chains in a relatively distinct region on one side of the protruding loop that joins β-strands 5 and 6 and is formed by residues K90 to T97 (Fig. 3). Electrostatic interactions between this region on profilin and PI(4,5)P_2 have been suggested by others [25,30], but K69 and K90 have not been identified as contributing to the interaction before. This result is congruent with the increased binding observed after deletion of P96 and T97, since deleting these residues must distort the protruding loop between K90 and T97, which in turn could enhance availability of K69 and K90 for the relatively bulky PI(4,5)P_2 micelles.

Residue K125 is located in the N-terminal part of the C-terminal helix, away from K69 and K90 and closer to the poly(L-proline) site. This residue contributes to actin-binding by forming a salt bridge with E364 in actin [29], stabilizing the interaction at the flank of the contact with actin subdomain I. The mutant profilin K125N expresses a tighter interaction with actin [6] but it is unclear how this is related to its reduced affinity for PIP_2. Another mutation introduced here, K125A, had the opposite effect on actin-binding [31], demonstrating the importance of this residue for the interaction with actin. The consequence for the PI(4,5)P_2 interaction was not reported in the latter case. Further away but still on the same side of the profilin molecule and central to the poly(L-proline)-binding site is residue W3 located (Fig. 3). The altered behavior in SDS–PAGE of W3N–profilin compared to the wild-type protein, and its inability to bind poly(L-proline) indicated a distorted conformation in the mutant protein [23,27]. Apparently this change in structure enhanced binding to PI(4,5)P_2, possibly reflecting an improved electrostatic interaction between the charged micelles and R135 and R136 in the nearby C-terminal helix. An interaction with PI(4,5)P_2 in this region of the molecule, e.g. in the nearness of the poly(L-proline) site is in agreement with several observations; Raghu-

nanthan et al. [32], and Lu et al. [16] observed a quenching of tryptophan fluorescence (residues 3 and 31) in the presence of $PI(4,5)P_2$, Sohn et al. [30] and Kovar et al. [33] reported altered $PI(4,5)P_2$-binding after mutating residue 8 in the closely positioned N-terminal helix, Chaudhary et al. [34] cross-linked the lipid moiety of a $PI(4,5)P_2$ analog to the N-terminal alanine and Lambrecht et al. [35] observed competition between $PI(4,5)P_2$ and poly(L-proline).

The overall structure of profilin from different species including plants is highly conserved [9,36–38] though rather extensive variations in amino acid sequences are at hand. Sequence alignment based on the structural analysis [36] shows that several residues which carry a positive charge in mammalian profilin are replaced with uncharged or negatively charged residues in the profilin isoforms 1 and 3 from *A. thaliana*. For instance R74, K90, K125, R135 and R136 in human profilin I are replaced with Q, G, L, I/L, and E, respectively, in the plant profilins (isoform 1 has an isoleucine at position 135 and isoform 3 a leucine). Binding studies with these plant profilins showed that both had a dramatically lower affinity for $PI(4,5)P_2$ than human profilin (Fig. 4). Since R74 appears to be excluded from the binding surface (Table 1) and positions 86, 88, and 89 in the plant profilins carry positive charges (R, K, K, compared to D, R, T in mammalian profilin), it seems that the replacements of the C-terminal residues 125, 135 and 136 are the main cause to the low affinity for $PI(4,5)P_2$ expressed by these profilins. The complete charge shift at position 136 (R136E) is perhaps the most important variation in this context. Again this supports the view that residues in the C-terminal helix contribute to the interaction. Similar charge shifts are present in a profilin from *Chlamydomonas*, which was reported to hardly bind $PI(4,5)P_2$ at all [39], and an aspartic acid at this position (R136D) in mammalian profilin was reported to reduce binding [40]. In our laboratory the profilin mutant R136I was expressed, for unknown reasons it could not be isolated in quantities large enough for a thorough analysis, but preliminary experiments (not shown) indicated that it was less efficient in binding $PI(4,5)P_2$ than the wild-type protein.

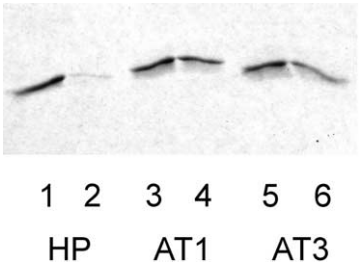

Fig. 4. Comparison of the $PI(4,5)P_2$-binding properties displayed by *A. thaliana* profilins 1 and 3 with human profilin I using the filter assay as in Fig. 2. The left lane in each pair shows the starting sample and the right the filtrate containing free profilin. Human profilin I, HP; *A. thaliana* profilins 1 and 3, AT1 and AT3, respectively.

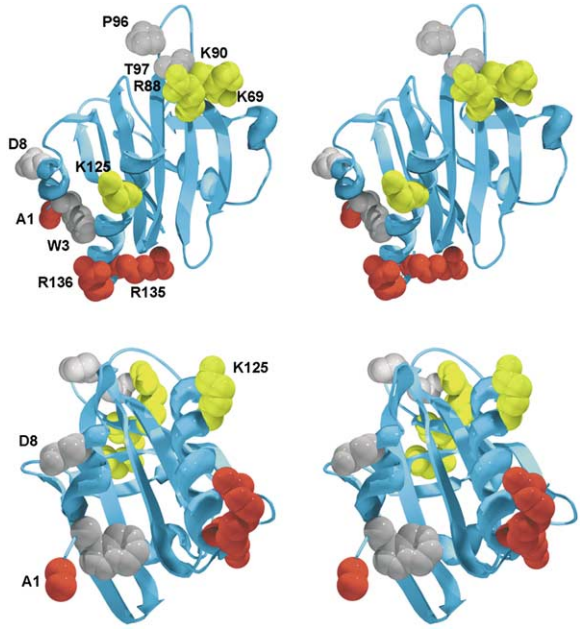

Fig. 3. Ribbon structure of profilin I shown in stereo in two orientations. Amino acid residues discussed in the paper are displayed as space-filled objects. Those involved in binding to actin and proposed to represent one of the two $PI(4,5)P_2$-interacting clusters on profilin (K69, R88, K90) are shown in yellow color, P96, T97, D8 and W3 appear white and residues depicted as important for the second electrostatic interaction surface (R135, R136) are red together with the residue (A1) that was cross-linked to a $PI(4,5)P_2$ analog in [34]. Note that the second interaction is separated from the actin-binding surface profilin:actin; K125 takes an intermediary position between the two charged patches. Images were prepared using SwissPdb Viewer ([43] http://www.expasy.ch/spdbv/ and POV-Ray (http://www.povray.org.).

The results with the point mutations are presented in Table 1 together with a summary of literature data. Clearly two separate locations for interaction with $PI(4,5)P_2$ on mammalian profilin are indicated; one partially overlapping with the actin site and another located in the nearness of the proline-binding surface (Fig. 3). This is in agreement with the fact that PxA was observed to bind $PI(4,5)P_2$. Perhaps binding of $PI(4,5)P_2$ micelles to profilin:actin proceeds via successive interactions, initiated by hydrophobic and electrostatic contacts at the N- and C-terminal region and involving R135, R136, and subsequently K125 before actin is released and further electrostatic association with K69, R88 and K90 becomes possible. It is interesting that the identity of the lipid moiety also influences binding affinity as reported by Chaudhary et al. [34], who suggested that this part of the molecule via hydrophobic interactions in the region of the poly(L-proline)-binding site contributes to orienting the bis-phosphorylated inositol ring towards the positively charged side chains of R135 and R136. Perhaps this hydrophobic interaction is reflected in the quenching of tryptophan fluorescence [32]. However, it is not clear how the binding of this $PI(4,5)P_2$ analog in non-micellar form compares to that of $PI(4,5)P_2$ micelles, which bury their hydrophobic part and therefore primarily must contact the protein via electrostatic interactions.

It is noteworthy that a location for $PI(4,5)P_2$-binding to the surface formed by the N- and C-terminal helices positions the interaction in an area of the molecule that is directly involved in the binding to proline-containing partners, and via the acetylated alanine in the N-terminus, forms a contact with actin. This actin contact has been proposed to mediate a switch function, pivotal in the incorporation of actin from profilin:actin during actin polymerization [9]. Maybe formation of a stable PxA–$PI(4,5)P_2$ complex reflects a mechanism for accumulation of polymerizable actin in a dynamic form at

$PI(4,5)P_2$-rich sites, e.g. lipid rafts [41,42], on the inner leaflet of the plasma membrane.

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References

[1] Carlsson, L., Nyström, L.E., Sundkvist, I., Markey, F. and Lindberg, U. (1977) J. Mol. Biol. 115, 465–483.
[2] Markey, F., Persson, T. and Lindberg, U. (1981) Cell 23, 145–153.
[3] Markey, F., Larsson, H., Weber, K. and Lindberg, U. (1982) Biochim. Biophys. Acta 704, 43–51.
[4] Pollard, T.D. and Cooper, J.A. (1984) Biochemistry 23, 6631–6641.
[5] Pring, M., Weber, A. and Bubb, M.R. (1992) Biochemistry 31, 1827–1836.
[6] Korenbaum, E., Nordberg, P., Björkegren-Sjögren, C., Schutt, C.E., Lindberg, U. and Karlsson, R. (1998) Biochemistry 37, 9274–9283.
[7] Kang, F., Purich, D.L. and Southwick, F.S. (1999) J. Biol. Chem. 274, 36963–36972.
[8] Nyman, T., Page, R., Schutt, C.E., Karlsson, R. and Lindberg, U. (2002) J. Biol. Chem. 277, 15828–15833.
[9] Cedergren-Zeppezauer, E.S., Goonesekere, N.C., Rozycki, M.D., Myslik, J.C., Dauter, Z., Lindberg, U. and Schutt, C.E. (1994) J. Mol. Biol. 240, 459–475.
[10] Hajkova, L., Nyman, T., Lindberg, U. and Karlsson, R. (2000) Exp. Cell. Res. 256, 112–121.
[11] Carlsson, L., Markey, F., Blikstad, I., Persson, T. and Lindberg, U. (1979) Proc. Natl. Acad. Sci. USA 76, 6376–6380.
[12] Schluter, K., Jockusch, B.M. and Rothkegel, M. (1997) Biochim. Biophys. Acta 1359, 97–109.
[13] Lindberg, U., Schutt, C.E., Hellsten, E., Tjäder, A.C. and Hult, T. (1988) Biochim. Biophys. Acta 967, 391–400.
[14] Lassing, I. and Lindberg, U. (1985) Nature 314, 472–474.
[15] Sohn, R.H. and Goldschmidt-Clermont, P.J. (1994) Bioessays 16, 465–472.
[16] Lu, P.J., Shieh, W.R., Rhee, S.G., Yin, H.L. and Chen, C.S. (1996) Biochemistry 35, 14027–14034.
[17] Ostrander, D.B., Gorman, J.A. and Carman, G.M. (1995) J. Biol. Chem. 270, 27045–27050.
[18] Hartwig, J.H., Bokoch, G.M., Carpenter, C.L., Janmey, P.A., Taylor, L.A., Toker, A. and Stossel, T.P. (1995) Cell 82, 643–653.
[19] Tall, E.G., Spector, I., Pentyala, S.N., Bitter, I. and Rebecchi, M.J. (2000) Curr. Biol. 10, 743–746.
[20] Sechi, A.S. and Wehland, J. (2000) J. Cell Sci. 113, 3685–3695.
[21] Lassing, I. and Lindberg, U. (1988) J. Cell Biochem. 37, 255–267.
[22] Aspenström, P., Lassing, I. and Karlsson, R. (1991) J. Muscle Res. Cell Motil. 12, 201–207.
[23] Björkegren-Sjögren, C., Korenbaum, E., Nordberg, P., Lindberg, U. and Karlsson, R. (1997) FEBS Lett. 418, 258–264.
[24] Rozycki, M., Schutt, C.E. and Lindberg, U. (1991) Methods Enzymol. 196, 100–118.
[25] Haarer, B.K., Petzold, A.S. and Brown, S.S. (1993) Mol. Cell. Biol. 13, 7864–7873.
[26] Sugiura, Y. (1981) Biochim. Biophys. Acta 641, 148–159.
[27] Björkegren, C., Rozycki, M., Schutt, C.E., Lindberg, U. and Karlsson, R. (1993) FEBS Lett. 333, 123–126.
[28] Hajkova, L., Björkegren Sjögren, C., Korenbaum, E., Nordberg, P. and Karlsson, R. (1997) Exp. Cell. Res. 234, 66–77.
[29] Schutt, C.E., Myslik, J.C., Rozycki, M.D., Goonesekere, N.C. and Lindberg, U. (1993) Nature 365, 810–816.
[30] Sohn, R.H., Chen, J., Koblan, K.S., Bray, P.F. and Goldschmidt-Clermont, P.J. (1995) J. Biol. Chem. 270, 21114–21120.
[31] Schluter, K., Schleicher, M. and Jockusch, B.M. (1998) J. Cell Sci. 111, 3261–3273.
[32] Raghunathan, V., Mowery, P., Rozycki, M., Lindberg, U. and Schutt, C. (1992) FEBS Lett. 297, 46–50.
[33] Kovar, D.R., Drøbak, B.K., Collings, D.A. and Staiger, C.J. (2001) Biochem. J. 358, 49–57.
[34] Chaudhary, A., Chen, J., Gu, Q.M., Witke, W., Kwiatkowski, D.J. and Prestwich, G.D. (1998) Chem. Biol. 5, 273–281.
[35] Lambrechts, A., Verschelde, J.L., Jonckheere, V., Goethals, M., Vandekerckhove, J. and Ampe, C. (1997) EMBO J. 16, 484–494.
[36] Thorn, K.S., Christensen, H.E., Shigeta, R., Huddler, D., Shalaby, L., Lindberg, U., Chua, N.H. and Schutt, C.E. (1997) Structure 5, 19–32.
[37] Almo, S.C., Pollard, T.D., Way, M. and Lattman, E.E. (1994) J. Mol. Biol. 236, 950–952.
[38] Fedorov, A.A., Ball, T., Mahoney, N.M., Valenta, R. and Almo, S.C. (1997) Structure 5, 33–45.
[39] Kovar, D.R., Yang, P., Sale, W.S., Drobak, B.K. and Staiger, C.J. (2001) J. Cell Sci. 114, 4293–4305.
[40] Lambrechts, A. et al. (2000) Mol. Cell. Biol. 20, 8209–8219.
[41] Pike, L.J. and Casey, L. (1996) J. Biol. Chem. 271, 26453–26456.
[42] Liu, Y., Casey, L. and Pike, L.J. (1998) Biochem. Biophys. Res. Commun. 245, 684–690.
[43] Guex, N. and Peitsch, M.C. (1997) Electrophoresis 18, 2714–2723.
[44] Ostrander, D.B., Ernst, E.G., Lavoie, T.B. and Gorman, J.A. (1999) Eur. J. Biochem. 262, 26–35.