

The primary structure of the basic isoform of *Acanthamoeba* profilin

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Acanthamoeba profilin-II [Kaiser, D. A., Sato, M., Ebert, R. F. and Pollard, T. D. (1986) *J. Cell. Biol.* 102, 221–226] was digested with trypsin or cleaved by 2-(2-nitrophenylsulphenyl)-3-methyl-3-bromoindolenine. The tryptic peptides were purified by reversed-phase-high-performance liquid chromatography and completely sequenced using automated gas-phase sequence analysis. The complete profilin-II sequence was deduced by ordering the tryptic peptides using the sequence information of the tryptophan-cleavage products. *Acanthamoeba* profilin-II was found to be homologous to the previously determined profilin-I sequence [Ampe, C., Vandekerckhove, J., Brenner, L., Tobacman, L. and Korn, E. D. (1985) *J. Biol. Chem.* 260, 834–840]. Like profilin-I, profilin-II consists of 125 amino acids, has a blocked NH₂ terminus and a trimethyllysine residue at position 103. Profilin-II differs in at least 21 positions from one of the profilin-I isoforms. The amino acid exchanges are mainly concentrated in the middle part of the sequence. Profilin-II contains two more basic residues than profilin-I, which explains its higher isoelectric point.

Profilin, a small cytoplasmic protein was first isolated from calf spleen as a 1:1 complex with actin (profilactin) [1]. Since then, profilin or profilin-like proteins were discovered in a variety of mammalian non-muscle tissues or cells (e.g. thymus [2], brain [2, 3], thyroid [4], platelets [5, 6], macrophages [7] and in some invertebrate cells (sea-urchin egg [8], *Thyone* sperm [9]). Proteins with similar properties were also purified from *Physarum* slime molds [10] and from *Acanthamoeba* [11]. In the latter organism, profilin was found in high concentrations in the cytoplasm [12].

Originally the function of profilin was thought to keep a pool of unpolymerized actin in cells by binding to and sequestering monomeric actin [1]. Recently a more complex mechanism of regulation of actin filament formation was proposed [9, 13]. In particular, electron-microscopic data suggested that *Acanthamoeba* profilin inhibits elongation of the actin filament by capping the barbed end [13] although it has not yet been possible to confirm this effect in experiments with bulk samples [14]. Vertebrate profilin may share some of these features with *Acanthamoeba* profilin (reviewed in [15]). Both calf spleen profilin [16] and *Acanthamoeba* profilin have been sequenced. The latter consists of a mixture of two closely similar isoforms (referred to as profilin-I a/b) [17]. The calf spleen protein is a basic protein (pI ± 9.3) of 142 amino acids, while both forms of *Acanthamoeba* profilin-I are more acidic (pI 5.5) and only 125 amino acids long. The overall sequence homology between calf spleen and *Acanthamoeba* profilin is low, but the NH₂-terminal parts of both proteins are similar. This suggests that the NH₂-terminal part of profilin is important in its interaction with actin [17].

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Abbreviation. BNPS-skatole, 2-(2-nitrophenylsulphenyl)-3-methyl-3-bromoindolenine.

Enzymes. Trypsin (EC 3.4.21.4); carboxypeptidase Y (EC 3.4.16.1).

Recently Kaiser et al. reported the presence of a third profilin isoform in *Acanthamoeba* [18]. This form (referred to as profilin-II) has the same molecular mass as profilins-Ia/b, and inhibits actin polymerization in a similar manner. In addition profilin-I and profilin-II share at least one antigenic site. On the other hand profilin-II has a much higher isoelectric point (9.0) [18] than profilin-I and the tryptic peptide patterns on a HPLC-C₄ reversed-phase column were reported to be 'substantially different' [18].

We have determined the complete amino acid sequence of profilin-II. It differs by about 20% from both profilin-Ia and Ib, with most of the substitutions in the middle third of the molecule.

MATERIALS AND METHODS

Protein and peptide separation

Acanthamoeba profilin-II was purified as in [18] and digested with trypsin as described for profilin-I [17]. Tryptic digestions were terminated by diluting the reaction mixture ten times with 0.1% trifluoroacetic acid. Peptides were separated by reversed-phase HPLC over a Vydac C₄ column (Separations group, Hesperia, USA) using a gradient of 0.1% trifluoroacetic acid to 70% acetonitrile in 0.1% trifluoroacetic acid [19]. Gradients were as indicated in the figures. The HPLC equipment consisted of two 510 pumps, a U6K injector, a 660 data controller and a lambda max 410 detector (all from Waters Assoc., Millipore Inc., USA) and a SE 120 BBC recorder (Goerz, Austria). The flow rate was kept at 1 ml/min and absorbance was measured at 214 nm. Fractions were collected by hand. Peptides not retained by the C₄ reversed-phase column were rechromatographed on a Vydac C₁₈ column under the same gradient conditions as described above.

Cleavage of profilin-II with 2-(2-nitrophenylsulphenyl)-3-methyl-3-bromoindolenine (BNPS-skatole) [20] was carried

out in 88% acetic acid for 24 h at 20°C. Excess of reagent was removed by extracting the lyophilized mixture with acetone/acetic acid/triethylamine/water (17:1:1:1, by volume). One fragment was extracted with pH 6.5 buffer (10% pyridine, 0.5% acetic acid) at 4°C, while the larger fragment remained insoluble [17].

Amino acid analysis

Amino acid hydrolysis was carried out using standard conditions (e.g. see [17]) and amino acids were quantified as phenylthiocarbonyl derivatives by HPLC [21]. Phenylthiocarbonyl-trimethyllysine elutes in this system in front of phenylthiocarbonyl-arginine. The presence of tryptophan was assigned by spot test. Therefore 10% of each peptide was applied as a small spot on Whatmann 1 MM paper and stained for tryptophan using *p*-dimethylaminobenzaldehyde/HCl [22].

Amino acid sequence determinations

The COOH-terminal sequence was deduced by time-course amino acid analysis of a carboxypeptidase Y (Boehringer Mannheim, FRG) digestion on 3 nmol profilin-II. Conditions were as described [23]. Aliquots were analyzed after 0, 30 and 120 min. Amino acid sequence analyses were carried out with a gase-phase sequencer (type 470A, Applied Biosystems, USA) [24] and the stepwise liberated amino acids phenylthiohydantoin were identified as described previously [25]. The phenylthiohydantoin derivative of trimethyllysine elutes behind the arginine derivative. In one case (for peptide N30) the on-line phenylthiohydantoin analysis system was used (120A, Applied Biosystems Inc., USA).

Peptide nomenclature

Tryptic peptides are indicated with T. They are numbered according to the position of their NH₂-terminal and COOH-terminal residue in the sequence. The tryptophan-cleavage fragments are designated with N and numbered by the position of their NH₂-terminal residue.

RESULTS AND DISCUSSION

The amino acid composition of profilin-II is very similar to that derived from the previously determined profilin-I sequence (Table 1). Note that profilin-II also contains a trimethyllysine residue and one Lys and one Arg (potential tryptic cleavage sites) more than profilin-I.

An attempt at Edman degradation of intact profilin-II demonstrated that, like *Acanthamoeba* profilin-Ia/b and calf spleen profilin, the NH₂ terminus is blocked. The nature of the blocking group was not investigated but is assumed to be an acetyl group, like calf spleen profilin [16].

Amino acids which are mainly released from the carboxypeptidase Y digest are listed in Table 2. The relatively high value of serine present in the blank sample does not vary during prolonged digestion and is probably due to contamination in the sample. Phenylalanine is preferentially released, followed by glycine and to a lesser extent by glutamine. These results are consistent with a COOH-terminal sequence -Gln-Gly-Phe, in accordance with the COOH-terminal sequence of peptide T116-125 and profilin-I a/b (see below).

Table 1. Amino acid composition of *Acanthamoeba* profilin-II (PII) (a) Calculated from a 24-h acid hydrolysate (average of two independent hydrolysates); (b) taken from the sequence (see Fig. 2). The amino acid composition of *Acanthamoeba* profilin-I (PI) is taken from [17]. n.d., not determined

Amino acid	(a) PII	(b) PII	PI
Cys	—	—	—
Asx	13.4	13	13
Thr	11.3	12	11.5
Ser	7.3	7	5.5
Glx	8.1	7	7.5
Pro	2.0	2	2.5
Gly	14.3	15	16.5
Ala	19.9	20	18.5
Val	10.1	11	12
Met	—	—	—
Leu	6.3	6	9
Ile	7.6	9	7
Tyr	4.8	5	5
Phe	4.0	4	5
Lys	5.7	6	5
His	1.1	1	1
Arg	3.8	4	3
Trp	n.d.	2	2
Me ₃ -Lys	+	1	1

Table 2. Time course amino acid analysis of a carboxypeptidase Y digest on 3 nmol profilin-II. At the indicated times aliquots were taken and analyzed as described in Materials and Methods

Amino acid	Time (min)		
	0	30	120
	nmol		
Gly	0.3	0.4	0.6
Phe	—	0.3	0.6
Gln	—	0.1	0.2
Ile	—	—	0.1
Leu	—	—	0.1
Ser	0.2	0.2	0.1

Profilin-II was digested with trypsin or cleaved with BNPS-skatole, a strategy which was found useful in the elucidation of the primary structure of profilin-I [17]. Instead of separating the tryptic peptides of profilin-II in two dimensions on paper, as we did for profilin-I [17], we used the more sensitive HPLC peptide mapping technique on C₄ or C₁₈ reversed-phase columns [19] (Fig. 1). The peptides were characterized by both their amino acid composition (Table 3) and full amino acid sequences (Fig. 2). One-third of the amount given in Table 3 was used for either analysis. All of the profilin-II tryptic peptides, except those derived from the blocked NH₂ terminus, could be sequenced completely. No microheterogeneity was observed. The initial coupling yields varied from 26% to 42% and repetitive yields of reliably measurable residues were at least 92%. These peptides were aligned by the sequences obtained from the tryptophan-cleavage fragments. In our previous studies on the profilin-I sequence we were unable to obtain sequence information from the short fragment recovered by extraction with pH 6.5 buffer

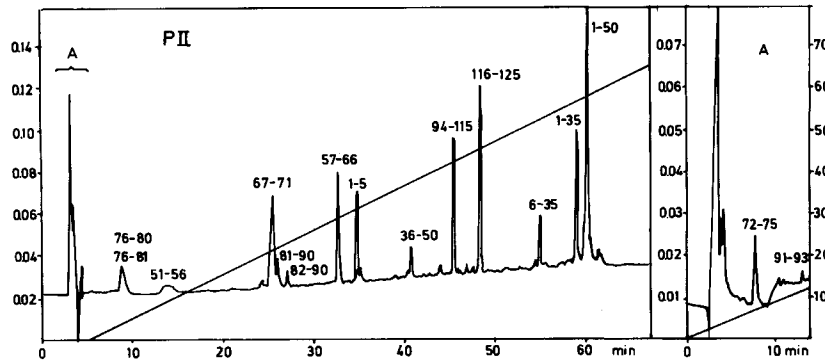


Fig. 1. Reversed-phase HPLC separation patterns of tryptic peptides derived from *Acanthamoeba profilin-II* (PII). Left: separation pattern on a C4 column. Peptides not retained by the C4 column (fraction A) were applied onto a C18 column (right). Detection is at 214 nm (absorption units full scale, left ordinate). Peptides are numbered according to their position in the sequence (see Fig. 2). Gradients are indicated (%B, right ordinate)

Table 3. Amino acid composition of the tryptic peptides of *Acanthamoeba profilin-II*

The peptides are numbered according to their position in the sequence. Peptide T76–81 is a mixture of peptides T76–80 and T76–81. Under each column the purified quantities of the peptide is given. The presence of tryptophan was assigned by a spot test using dimethylaminobenzaldehyde/HCl [22]

	1–50	1–5	6–35	36–50	51–56	57–66	67–71	72–75	76–81	81–90	82–90	91–93	94–115	116–125
Cys	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Asx	6.0	–	4.1	2.0	0.9	0.9	–	1.9	–	–	–	–	1.9	0.9
Thr	6.0	0.9	4.6	–	0.9	1.0	0.9	–	–	0.9	0.8	0.9	0.9	–
Ser	2.9	0.9	0.9	0.9	–	0.9	–	–	0.9	0.9	0.9	0.9	–	–
Glx	1.9	1.0	1.0	–	–	1.0	–	–	–	–	–	–	3.1	0.9
Pro	1.2	–	–	1.1	–	–	–	–	–	–	–	–	0.9	–
Gly	6.0	–	5.3	1.2	–	2.0	–	–	1.1	2.0	2.2	–	2.2	2.1
Ala	10.7	–	5.1	6.2	1.9	1.1	–	1.1	–	1.1	1.0	–	3.0	1.0
Val	4.1	–	3.0	1.1	–	–	1.2	–	1.1	1.8	1.9	–	2.7	–
Met	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Leu	2.1	–	1.1	1.0	–	1.0	–	–	–	–	–	–	1.3	2.2
Ile	1.7	–	1.8	–	1.2	–	1.2	–	–	1.2	1.0	–	3.3	1.0
Tyr	0.9	1.1	–	–	–	–	0.8	–	1.0	–	–	–	1.2	1.0
Phe	2.0	–	1.0	0.9	–	1.1	–	–	–	–	–	–	–	1.0
Lys	0.9	–	–	0.9	–	–	–	–	1.5	1.9	1.2	1.1	0.9	–
His	1.2	–	1.1	–	–	–	–	–	–	–	–	–	–	–
Arg	–	–	–	–	1.1	1.0	0.8	1.0	–	–	–	–	–	–
Trp	+	+	+	–	–	–	–	–	–	–	–	–	–	–
Me ₃ -Lys	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Content (nmol)	8	15	5	8	16	18	19	15	20	12	10	12	19	20

(residues 3–29). This was explained by the conversion of the NH₂-terminal glutamine residue (position 3 in the sequence) into 5-pyrrolidone-2-carboxylic acid due to the buffer extraction. By carrying out the same extraction procedure at 4°C, we were now able to limit the degree of glutamine conversion, so that it was possible to obtain a partial NH₂-terminal sequence (see Fig. 2). This was sufficient to align N3 with peptide T6–35. The amino-acid composition of T1–5 (a tryptophan-containing peptide generated by partial non-specific cleavage at the tyrosine residue on position 5) and the specificity of the BNPS-skatole reagent allow us to order the amino acids at the NH₂ terminus of profilin-II as blocked-Thr-Trp-Gln-Ser-, identical to profilin-I [17] (Fig. 2).

The insoluble large tryptophan-cleavage fragment N30 could be sequenced with high efficiency. In a first run 11 residues could be identified. A second run performed on

5 nmol, now using the on-line amino acid phenylthiohydantoin analyzer, yielded a sequence of 67 residues, extending the profilin sequence from residue 30 through 96 and providing the alignment of peptides T6–35, T36–50, T51–56, T57–66, T67–71, T72–75, T76–81 and T82–90. The residues at positions 83, 88, 91 and 92 could not be assigned unambiguously. The last identifiable residues were Ala-Ile-Leu which allowed us to position peptide T94–115.

Of the two remaining peptides, peptide T116–125 could be located at the COOH-terminal end of the protein, since it contains the profilin-II COOH-terminal sequence (Gln-Gly-Phe) while the remaining tripeptide T91–93 could be positioned at residues 91–93. The position of both peptides also agrees with the sequence of profilin-I (see below). Note that the gaps (positions 83, 88, 91 and 92) are now filled up by either serine or threonine. These amino acids are difficult

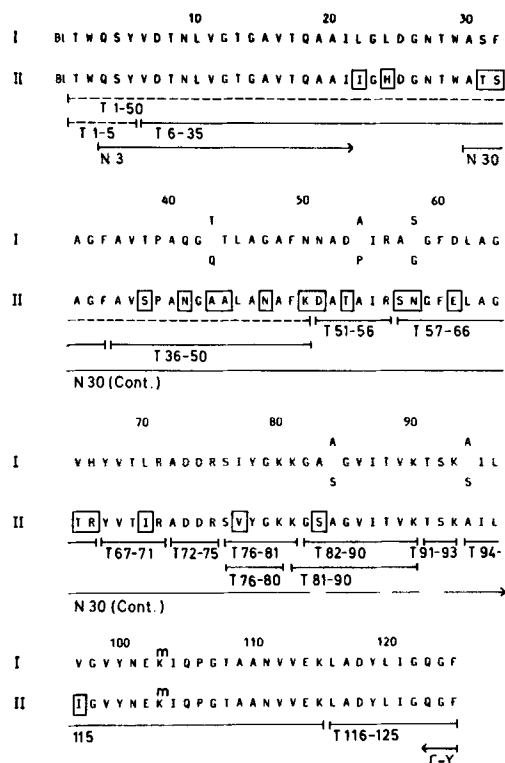


Fig. 2. The complete amino acid sequence of *Acanthamoeba* profilin II: comparison with profilin-I. The tryptic peptides (T) are characterized by their full-length sequence (—), except for T1–5 and T1–50 containing a blocked NH₂ terminus (---). The sequenced stretches of the Trp-cleavage fragments (N) are underlined. In N30 the residues at positions 83, 88, 91 and 92 could not be identified (gaps). The COOH-terminal sequence was deduced using carboxypeptidase-Y (C–Y). The sequence of profilin-II (II) was aligned to the one of profilin-Ia/b (I). Amino acid exchanges with regard to profilin-Ia/b are boxed. K^m at position 103 stands for trimethyllysine, Bl indicates the blocking group. Note that the profilin-I sequence shows two amino acid residues at positions 43, 54, 58, 84 and 94

to identify since they are known to be converted with low yield into their corresponding phenylthiohydantoin derivatives.

Like profilin-I, profilin-II also consists of 125 amino acids ($M_r = 12963$) with a trimethylated lysine at position 103. At least 21 and at most 23 residues are exchanged with regard to either one of the two profilin-I isoforms. The homology is at least 81.6% and at most 83.2%, while the homology between profilin-Ia and Ib is 94%. At four positions there is an exchange of a neutral for a charged residue (positions 24, 50, 51 and 53 are respectively Leu, Asn, Asn and Asp in profilin-I and His, Lys, Asp and Thr in profilin-II). Profilin-II has two more basic amino acids than profilin-I, explaining its higher isoelectric point [18].

Most of the differences between profilins-I and II are in the middle part of the molecule (between positions 31 and 70), where the homology is only 60%, while the NH₂-terminal (residues 1–30) and the COOH-terminal parts (residues 71–125) are only slightly different with about 94% homology.

These features of the sequence suggest that profilin may consist of three functional domains: first, a weakly charged NH₂-terminal part which, on the basis of sequence homology between profilins from highly diverged species, may be in-

involved in the binding to actin [17]; second, a middle part, rich in alanine and, as already mentioned, the most variable region when *Acanthamoeba* profilin-I and II are compared. It is most likely that the middle section is not directly involved in actin binding since both profilin-I and II are known to have the same effect on actin polymerization *in vitro* [18]. This part could, however, be involved in a specialized interaction or regulation of profilin *in vivo* thereby differentiating the functions of profilin-I and II in the cell. Third, there is the COOH-terminal part, rich in basic amino acids, which also contains the trimethyllysine residue. Lassing and Lindberg reported the interaction of calf spleen profilin with phosphatidylinositol 4,5-bisphosphate, thus suggesting a possible link between the phosphatidylinositol cycle and actin filament formation in the cell [26]. Whether a similar interaction exists with *Acanthamoeba* profilin is not known. Yet it is tempting to speculate that some of the basic amino acids present in the COOH-terminal region might be involved in this kind of interaction.

Acanthamoeba expresses at least three isoforms of profilin: two neutral variants (profilin-Ia and Ib) and one more basic type (profilin-II). So far *Acanthamoeba* is the only organism in which multiple profilin isoforms have been found by protein-chemical means. Whether this is exceptional remains to be clarified by future experiments.

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