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The primary structure of human platelet profilin: reinvestigation of the calf spleen profilin sequence

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The primary structure of human platelet profilin was determined by aligning the sequences of its tryptic peptides to the previously determined calf spleen profilin sequence [(1979) FEBS Lett. 101, 161–165]. Comparison of the peptide fingerprints of the two proteins suggested a higher homology than that found by direct sequence comparison. We therefore reinvestigated the sequences of the peptides from calf spleen profilin. We identified four incorrect charge assignments and a deletion of three residues. The similarity between the two vertebrate profilins amounts to 95%.

Profilin; Amino acid sequence; (Calf spleen, Human platelet)

1. INTRODUCTION

The small actin-binding protein, profilin, has been isolated from a variety of sources including different mammalian non-muscle tissues or cell types [1–7], echinoderms [8,9], *Physarum* [10] and *Acanthamoeba* [11,12]. It inhibits the polymerization of actin and is therefore thought to function as an actin sequestering protein in vivo [1,13,14]. For *Acanthamoeba* and sea urchin egg profilin, an additional capping activity of the barbed end of actin filaments has been suggested [9,15]. In resting platelets, actin seems to exist primarily in the unpolymerized form in complex with profilin [13,16]. During stimulation with thrombin 50–60% of this unpolymerized form is rapidly recruited for filament formation [17–20] a process which is accompanied by changes in the interaction between profilin and actin [16,21]. Recently, it has been shown that phosphatidylinositol 4,5-bisphosphate by binding to profilin causes the dissociation of the profilin-actin complex [22] suggesting that agonist-activated turnover in the phosphatidylinositol cycle somehow may be coupled to the recruitment of actin for polymerization. Phosphatidylinositol 4,5-bisphosphate has also been shown to interact with gelsolin [23]. During the early phase of platelet activation gelsolin is activated from an inactive, non-actin interacting form, to a form which is complexed with actin [21,24]. This suggests the presence of actin and phospholipid interaction sites of regulatory importance in both proteins.

So far, only the calf spleen profilin and the three isoforms of *Acanthamoeba* profilin have been sequenced [25–27]. Based on the limited similarity between these two profilins, the NH_2-terminal region (residues 1–35) was proposed to contain an actin-binding domain [26]. Recently, we also noticed that the COOH-terminal part of *Acanthamoeba* profilin contained a stretch of 16 amino acids (residues 104–119) displaying a high similarity with an internal sequence of the barbed end capping proteins, *Physarum* cap42(a) and human gelsolin (residues 94–109) [28]. Therefore, we proposed that *Acanthamoeba* profilin might have two actin-binding domains. First, a sequestering domain located in the NH_2-terminal part, shared with calf spleen profilin, and second, a capping domain

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in the COOH-terminal part, in common with the barbed end capping proteins. Calf spleen profilin does not contain such a similar sequence and it might not have a capping activity.

Human platelet profilin exhibits a chromatographic behaviour on phosphocellulose which is different from that of calf spleen profilin (unpublished). In view of this difference and of the restricted sequence similarity seen between gelsolin and *Acanthamoeba* profilin as mentioned above, the question arose whether human platelet profilin might be more similar to the *Acanthamoeba* profilin than profilin from calf spleen. We therefore performed a comparative sequence analysis of the two mammalian profilins. During this work we encountered possible errors in the sequence originally proposed for calf spleen profilin [25], which are reported here. From the final sequence comparison it is clear that the two mammalian profilins are closely similar.

2. MATERIALS AND METHODS

Human platelet profilin and calf spleen profilin were purified as described in [1,2]. The proteins were oxidized with performic acid [29] and subsequently dialyzed three times against water and once against NH_4HCO_3. Human platelet profilin (2 mg) and calf spleen profilin (3 mg) were cleaved with trypsin (Sigma) for 2 h at 37°C with an enzyme to substrate ratio of 1:50. The reaction was stopped by freeze-drying. Peptides were dissolved in pH 6.5 buffer (10% pyridine, 0.5% acetic acid) and separated with a two-dimensional technique on paper as described (e.g. [26]). Trp-containing peptides were identified by their fluorescence after performic acid oxidation. Peptides were detected with a dilute fluorescamine stain [30] and eluted with pH 6.5 buffer.

The blocked NH_2-terminal tryptic peptides of both profilins were further cleaved with chymotrypsin (Sigma) using the same conditions as for trypsin. The resulting subpeptides were purified as described above.

Non-oxidized human platelet profilin (0.5 mg) was treated with 2-(2-nitrophenylsulfenyl)-3-methyl-3'-bromoindolenine (BNPS-skatole) according to Fontana [31]. The reaction mixture was dried and the excess reagent was removed with a mixture of acetone/acetic acid/triethylamine/water (17:1:1:1, by vol.). The remaining precipitate was extracted with pH 6.5 buffer and the soluble peptide was subjected to sequence determination (see below).

One third of each peptide was taken for standard acid hydrolysis and analyzed with a Biotronik amino acid analyzer equipped with a fluorescence detector measuring the *O*-phthaldialdehyde reaction products [32]; as a result, proline was not detected. This amino acid was identified in the course of the sequence determination.

Another third of each peptide was subjected to sequence determination using a gas-phase protein sequencer (Applied Biosystems, Inc.) operated according to Hewick et al. [33]. Stepwise liberated phenylthiohydantoin (PTH) amino acids were identified as described [34] and the amount was measured using an integrative recorder. Gaps were encountered where cysteic acid was present in the sequence but this amino acid could be assigned by subtraction of the peptide sequence from the composition.

Prefixes P and S are given to distinguish the human platelet and calf spleen profilin peptides, respectively; T designates the trypsin peptides, and C the chymotrypsin subpeptides derived from the NH_2-terminal tryptic peptides. Peptides are numbered according to their order in the sequence. Calf spleen profilin has one lysine and consequently one tryptic peptide less than human platelet profilin. The number of this peptide has been left out in the calf spleen profilin peptide numbering. Peptides starting at the same position, but having a different COOH-terminal residue are designated by a suffix a, for the shorter, and b, for the longer peptide.

3. RESULTS AND DISCUSSION

Amino acid sequence determination of intact human platelet profilin failed to reveal PTH-amino acids, indicating that the protein was NH_2-terminally blocked. The nature of the blocking group was not investigated here, but is assumed to be an acetyl group as is the case for calf spleen profilin [25].

To determine the sequence, tryptic peptides from the performic acid-oxidized protein were generated. These peptides were separated by a combination of paper electrophoresis and chromatography. In a parallel experiment, the tryptic peptides of performic acid-oxidized calf spleen profilin were purified. A comparison of the two fingerprints shows that both proteins are very similar (fig.1). Only three peptides move differently (T3, T9, and T12) and human platelet profilin contains one peptide more (T10). All platelet peptides were characterized by their amino acid composition (not shown) and, except for the blocked peptide T1, by their full-length sequence. To verify the sequence at the blocked NH_2-terminus, the corresponding peptide was cleaved with chymotrypsin and the subpeptides were purified using electrophoresis at pH 6.5 (not shown). Two peptides were obtained. The Trp-containing peptide was still blocked while the longer one could be sequenced (fig.2). From the sequence of the soluble Trp cleavage fragment (see section 2) and the composition of the blocked NH_2-terminal peptide, we can deduce the NH_2-terminal sequence of profilin

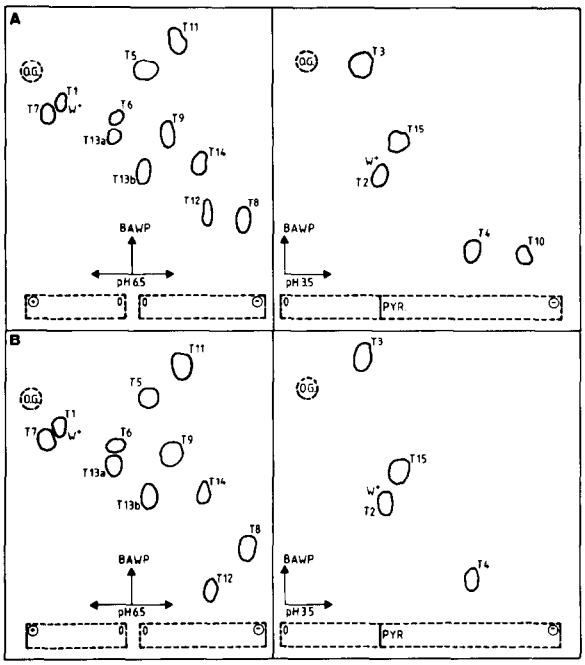

Fig.1. Two-dimensional separation on paper of the tryptic peptides of human platelet (A) and calf spleen profilin (B). High-voltage electrophoresis was carried out in the horizontal direction; at either pH 6.5 (left, acidic and basic peptides) or pH 3.5 (right, neutral peptides). Chromatography was carried out in vertical direction. (+) Anode; (−) cathode; O, origin; O.G. and PYR, dye markers Orange G and pyronin, respectively.

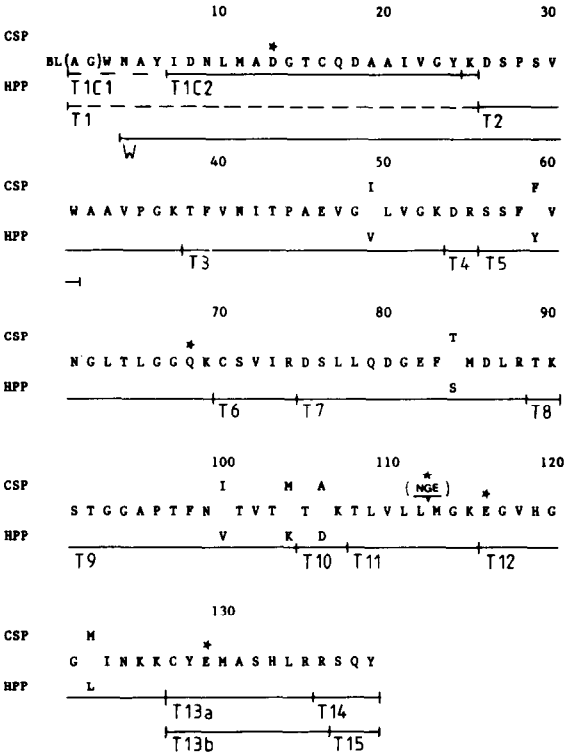

Fig.2. The amino acid sequence of calf spleen (CSP) and human platelet profilin (HPP). The platelet profilin peptides were characterized by their full-length sequence (full lines) except those containing a blocked NH_2-terminus (broken lines). W denotes the soluble tryptophan cleavage product. Differences between the two proteins are found at positions 49, 59, 84, 100, 104, 106 and 122. Residues denoted with an asterisk indicate the corrections made with regard to the calf spleen profilin sequence proposed in [25] (see text). The order of the first two amino acids in platelet profilin was not determined. These residues are positioned on the basis of the homology with spleen profilin and are shown between brackets.

as shown in fig.2. Similarity with the calf spleen profilin was sufficiently high so that the platelet profilin peptides could be readily aligned.

The soluble Trp cleavage fragment spanning region 4 to 31 showed one difference with the corresponding region in the previously published calf spleen profilin. At position 10 Asp was found instead of Asn. The same difference was found in T1C2. Peptides PT2, PT4, PT6, PT8, PT14 and PT15 were completely identical to their calf spleen profilin analogues, whereas peptides PT3, PT5, PT7, PT12, and PT13a and b were different from the published calf spleen counterparts. The additional peptide found in the human platelet profilin peptide map is due to the presence of lysine at position 104, generating the peptides PT9 and PT10, instead of ST9 as found in calf spleen profilin. Peptide PT11 appeared to be three residues shorter than ST11.

At this point our data disagree with the previously published sequence of calf spleen profilin [25]. The spleen profilin peptides ST1, ST5, ST11, ST12, and ST13a and b moved to similar positions during electrophoresis as their platelet profilin analogues. The electrophoretic mobility of these peptides, calculated according to Offord [35], differs from those predicted from the published sequence [25] (table 1). Also, if peptide ST11 contained the Asn-Gly-Glu sequence, then this should have markedly increased the retention during chromatography. Therefore, the sequence of these calf spleen profilin peptides was reinvestigated. Peptide ST1C2, generated and purified in a similar way to PT1C2, contained Asp at cycle 7 instead of Asn. At this point it should be

Table 1

Calculated charge of the basic and acidic tryptic profilin peptides at pH 6.5

Peptide	Position in the sequence	Calculated	Predicted from	
			This work	[25]
T1	1– 25	−3.70	−4	−3
T5	56– 69	+0.80	+1	0
T7	75– 98	−3.04	−3	−3
T8	89– 90	+0.94	+1	+1
PT9	91–104	+1.04	+1	+1
ST9	91–107	+1.01	+1	+1
T11	108–115	+0.95	+1	0
T12	116–126	+1.70	+1.5	+2.5
T13a	127–135	−0.40	−0.5	+0.5
T13b	127–136	+0.55	+0.5	+1.5
T14	136–139	+1.18	+1	+1

The charges were calculated by regression analysis using the relative mobilities determined according to Offord [35]. For cysteic acid and internal histidine a net charge of −1 and +0.5 was given, respectively

noted that Asn-Gly sequences are known to deamidate readily through hydrolysis of a cyclic imide formed in basic conditions and resulting in a mixture of α-aspartylglycine and β-aspartylglycine sequences [36]. Such a situation yields a mixture of Asp and Asn and reduces abnormally the repetitive yield of the Edman degradation at the position of the deamidated residue.

A quantitative estimation of the phenylthiohydantoin amino acid derivatives generated at positions 6, 7 and 8 during sequence analysis of peptide ST1C2 yielded 11.7, 9.9 and 8.1 pmol of the derivatives of Ala, Asp and Gly, respectively. In addition, no PTH-Asn was observed at position 7. We are therefore confident that the observed Asp at position 13 of the calf spleen profilin sequence is a true Asp residue rather than being derived by deamidation of an original Asn residue, as a comparison with the previously published sequence [25] might suggest.

Peptide ST5 had Gln instead of Glu at cycle 13 and peptides ST12, ST13a and b, contained Glu instead of Gln, at the first and third degradation cycle, respectively. These data are now in agreement with the calculated net-charges (table 1). Peptide ST11 was indeed three residues shorter than originally reported, consistent with its composition and its mobility in the chromatogram (table 1). All other tryptic calf spleen profilin peptides were sequenced but showed no difference with the previously published sequence. Thus, five corrections are proposed in the calf spleen profilin sequence as published previously. At positions 13, 68, 116 and 129, one should read Asp, Gln, Glu and Glu, instead of Asn, Glu, Gln, and Gln, respectively, and the tripeptide Asn-Gly-Glu (previously positions 113–115) should be deleted.

Calf spleen profilin is thus three residues shorter than originally reported and both profilins contain 139 amino aids. The true differences between calf spleen and human platelet profilin are then found at positions 49 (Ile-Val), 59 (Phe-Tyr), 84 (Thr-Ser), 100 (Ile-Val), 104 (Met-Lys), 106 (Ala-Asp), and 122 (Met-Leu) (fig.2). The similarity between the two proteins is thus 95%. Most of the differences found concern conservative amino acid exchanges, except at positions 104 (Met-Lys) and 106 (Ala-Asp). Although the isoelectric point of both proteins will still be very similar these changes induce a drastic difference in the hydrophobicity pattern covering region 98–113 (fig.3). This hydrophobic region is interrupted by two hydrophilic residues in human platelet profilin. Here, three-dimensional structure analysis will provide information on the impact of these amino acid exchanges on the overall structure and eventually on the actin interaction of both profilins.

The corrections made here do not significantly increase the similarity with the *Acanthamoeba* pro-

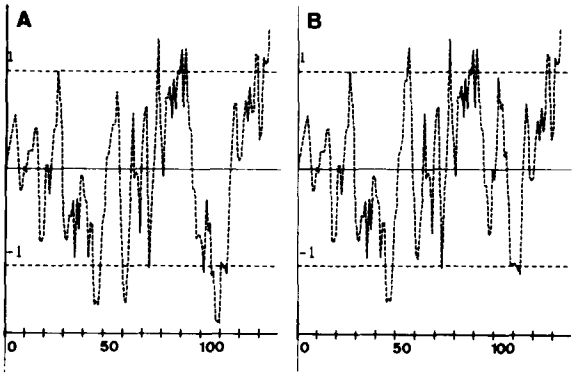

Fig.3. Comparison of the hydropathicity patterns of calf spleen profilin (A) and human platelet profilin (B). Profiles were calculated according to [38] using a span setting of 6 residues. Note the marked change in local hydropathy between residues 100 and 110.

filins [26,27]. However, it should be noted that no mutation has been found within the NH_2-terminal domain of the two vertebrate profilins. This region was proposed to participate in actin-binding, based on the limited similarity with *Acanthamoeba* profilin [26]. This region also remained very constant in the three *Acanthamoeba* profilin isoforms [27].

In the light of the possible similar active regulation mechanism of actin interaction with gelsolin or profilin in platelets [21] it is difficult to understand which sequence of both actin-binding proteins is involved. Indeed, no clear homologous region can be defined in the two proteins. The consensus sequence, as proposed by Burgoyne [37], found in gelsolin, p36 and p35, and thought to be involved in actin or phospholipid interaction is not readily recognized in the vertebrate profilins. It will be interesting to learn from the three-dimensional structure which region of vertebrate profilin is involved in phospholipid binding and whether a similar domain is present in gelsolin.

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