HUMAN PLATELETS CONTAIN PROFILIN, A POTENTIAL REGULATOR OF ACTIN POLYMERISABILITY

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1. Introduction

Profilin is a small basic protein (mol. wt 16 000) recently isolated in a 1:1 complex with actin from calf spleen. This complex, called profilactin, is resistant to polymerisation; moreover, profilin will bind to depolymerised rabbit skeletal muscle actin to form a polymerisation-resistant complex [1]. There is evidence for the existence of a profilin:actin complex in a variety of cell types, including human platelets [1]. Profilin may thus have an important regulatory influence on the state of actin polymerisation in non-muscle cells [1,2].

Ultrastructural evidence suggests that the stimulation of platelets during blood clotting involves extensive filament formation from initially unpolymerised actin [3–5]. We show here that human platelets indeed contain profilin, which closely resembles the corresponding protein from calf spleen. There is sufficient profilin in extracts of fresh platelets to inhibit the polymerisation of at least 55% total actin. An as yet hypothetical general scheme for the formation of actin filaments from profilactin was presented [1].

2. Materials and methods

Assay of DNAase I was as in [6] and SDS–polyacrylamide gel electrophoresis as in [7]. Viscosity was measured in a Cannon-Manning semi-micro capillary viscometer, requiring sample vol. 0.7 ml and having a flow time for buffer of about 60 s at 25°C.

The concentrations of purified proteins were determined from A_{280}, using extinction coefficients ($\epsilon^{1\%}_{1\ cm}$) of 11 for actin and 13 for profilin [1].

3. Results and discussion

Profilin was isolated from platelets obtained from platelet concentrate in plasma, supplied by Akademiska Sjukhuset, Uppsala. Platelets were washed 3 times at room temperature with phosphate-buffered saline pH 6.6, and the lower portion of the pellet, containing red and white blood cells, was discarded each time. Cells were then resuspended in 1 vol. 5 mM K_2HPO_4, pH 7.6, 0.1 mM $CaCl_2$, 0.5 mM ATP, 0.2 mM dithioerythritol (DTE) and sonicated on ice for 4 × 5 s. All subsequent operations were carried out at 0–4°C. The extract was centrifuged for 3.5 h at 100 000 × g and the supernatant was applied to a Sephadex G-100 column and eluted with 0.5 M K_2HPO_4, pH 7.6, 0.2 mM DTE (fig.1). Actin elutes both as an aggregate in the void volume of the column and as a peak identified by assay of DNAase I inhibition (see fig.1). This peak appears in the position expected for profilactin [1,7]. Coincident with the peak of unpolymerised actin, but absent from the void volume, is a protein which

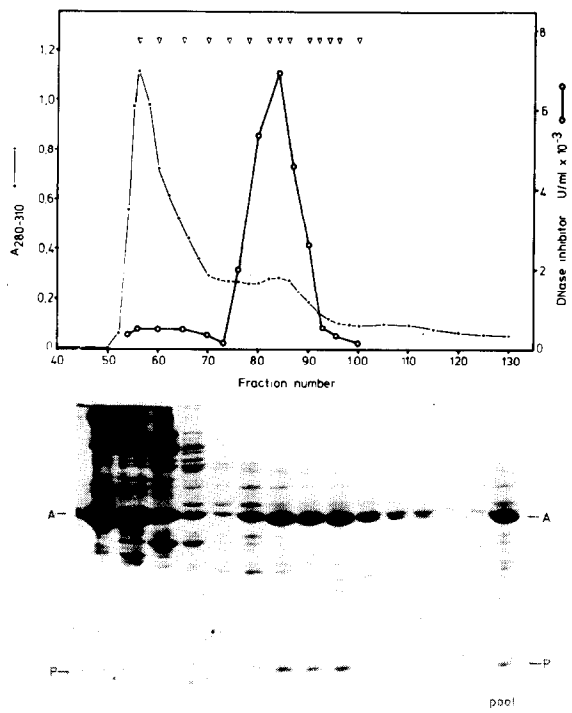

Fig.1. Chromatography of platelet extract on Sephadex G-100. About 12 ml packed platelets were sonicated in total vol. 35 ml buffer as described in the text. After centrifugation at 100 000 × g for 3.5 h, the supernatant, containing about 530 A_{280} units protein, was applied to a Sephadex G-100 column (5 × 115 cm) and eluted with 0.5 M K_2HPO_4, pH 7.6, 0.2 mM DTE at a flow rate of about 1 ml/min. Fractions (14 ml) were assayed for A_{280} (•——•) and for DNAase I inhibitor activity (○——○). The lower part of the figure shows SDS–gel analysis of the fractions marked (▽). The sample at the extreme right is the pooled material (fractions 74–92). The positions of actin (A) and profilin (P) from spleen profilactin are marked.

migrates close to calf spleen profilin on SDS gels.

Fractions containing DNAase I inhibitor activity were pooled and concentrated by precipitation with $(NH_4)_2SO_4$ at 80% saturation. The precipitated material was redissolved in and dialysed thoroughly against 25 mM Tris–HCl, pH 7.6, 5 mM $MgCl_2$, 0.2 mM DTE, 2 M urea, then passed through a DEAE-cellulose column (DE52) in the same buffer. These are conditions developed for the isolation of calf spleen profilin from profilactin [1]: profilin can be recovered from the flow-through fraction while actin is retained on the column. For the experiments described here, the material applied to the column is illustrated as the 'pool' in fig.1, while the unretarded fraction, which is enriched in the 16 000 dalton component, is shown as the 'start' material in fig.2.

The fraction not retained on DEAE-cellulose was precipitated with $(NH_4)_2SO_4$ at 85% saturation, redissolved in 5 mM K_2HPO_4 pH 7.6, 0.5 mM DTE, applied to a Sephadex G-75 column and eluted with the same buffer (fig.2). A_{280} located 2 peaks; the first eluted close to the void volume and was discarded. The second peak contained only the 16 000 dalton protein, as shown by gel electrophoresis of the pooled fractions. The pool was concentrated on a rotary evaporator to about 0.3 mg protein/ml, to facilitate subsequent precipitation with $(NH_4)_2SO_4$ at 85% saturation.

The association of the 16 000 dalton protein with unpolymerised actin in 0.5 M K_2HPO_4 (fig.1), its unretarded passage through DEAE-cellulose in 2 M urea, and its molecular weight together suggest that the protein isolated from platelets is profilin. This is confirmed by the experiment shown in fig.3. Different amounts of either the platelet protein or calf spleen profilin, the latter prepared from profilactin by the DEAE-cellulose–urea method [1], were mixed with a fixed amount of depolymerised rabbit skeletal muscle actin in 5 mM K_2HPO_4, pH 7.6, 0.1 mM $CaCl_2$, 0.1 mM ATP, 0.1 mM DTE. After incubation at 25°C for 10 min, NaCl and $MgCl_2$ were added to final conc 10 mM and 5 mM, respectively, and the actin polymerisation was followed by viscometry. The preparations from platelets and calf spleen cause equivalent inhibition of actin polymerisation. Further support for the identification of human platelet profilin comes from close similarity between the amino acid compositions of this protein and profilin from calf spleen (table 1) and from calf thymus and brain (unpublished observations).

The following experiment places the profilin content of platelets in perspective with respect to the actin content. Actin inhibits DNAase I by formation of a 1:1 complex [8], and this interaction is much more rapid with monomeric than with filamentous actin (unpublished observations; also [9]). Treatment with guanidine hydrochloride depolymerises filamentous actin but does not interfere with the actin–DNAase I interaction. We have recently developed a selective assay for monomeric and filamentous actin based on inhibition of DNAase I and utilising these

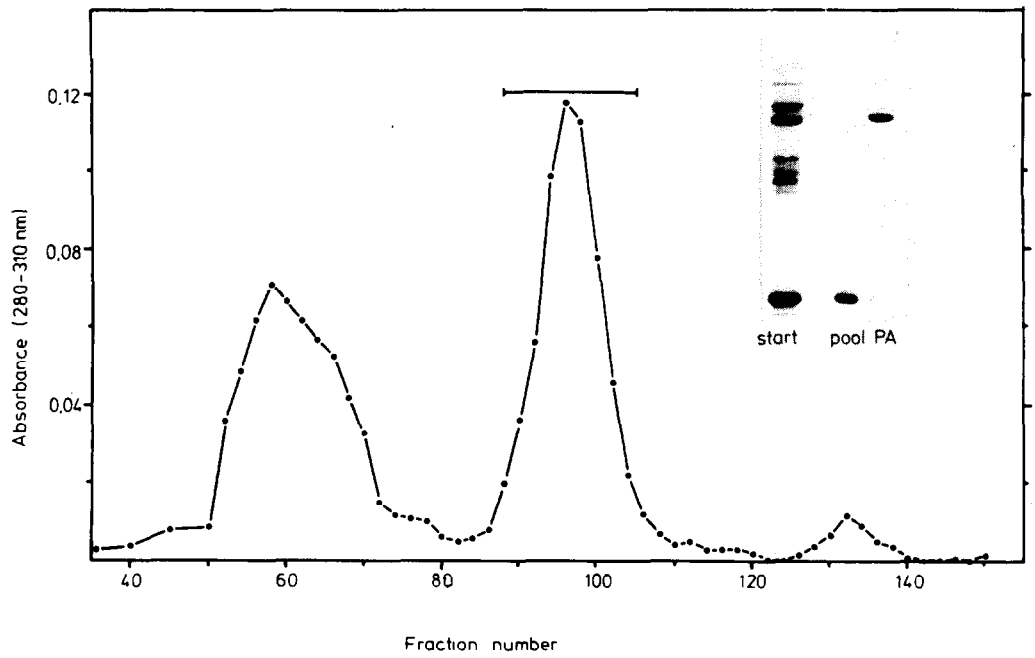

Fig.2. Chromatography of partly purified-platelet profilin on Sephadex G-75. Material not retained on DEAE-cellulose in 2 M urea (see text) was chromatographed on Sephadex G-75 in 5 mM K_2HPO_4, pH 7.6, 0.5 mM DTE. About 3.7 A_{280} units protein in 1 ml was applied to a 1.5 × 120 cm column and eluted at 0.25 ml/min. SDS–gel analysis of the starting material, the pool (marked by (⊢—⊣) on the chromatogram) and spleen profilactin (PA) are shown in the inset.

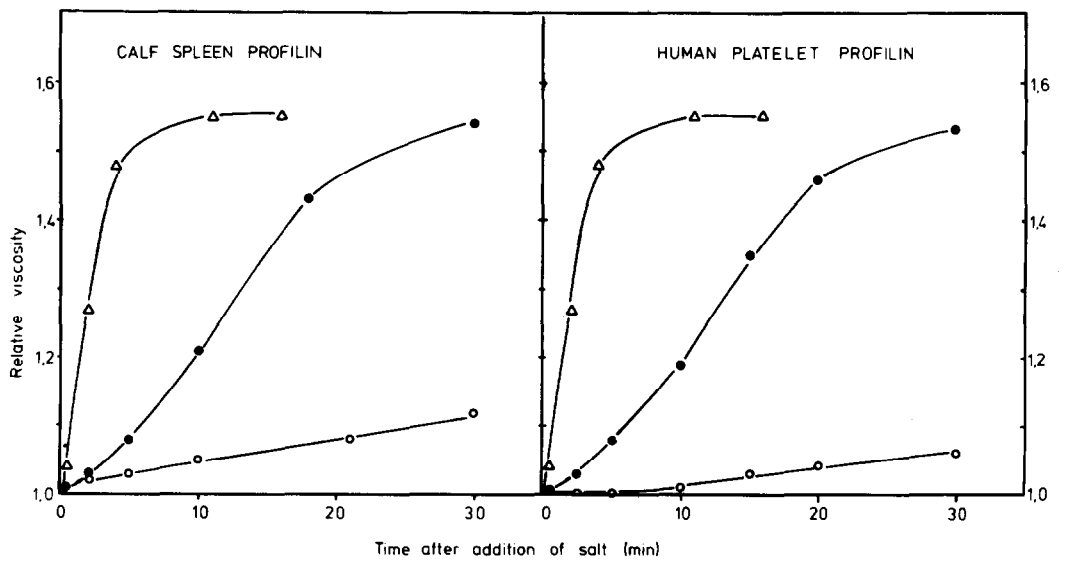

Fig.3. Recombination of profilin with muscle actin. Depolymerised rabbit skeletal muscle actin (0.29 mg) was mixed with buffer (△) or with either platelet or calf spleen profilin in stoichiometric ratio to profilin 1:0.46 (●) and 1:0.92 (○). After incubation for 10 min at 25°C, NaCl and $MgCl_2$ were added to 10 mM and 5 mM, respectively, and actin polymerisation was followed by viscometry.

Table 1
Amino acid composition of profilin from calf spleen and human platelets

	Calf spleen	Human platelets
Aspartic acid	15	15–16
Threonine	12–13	12
Serine	10–11	12
Glutamic acid	12	10
Proline	6– 7	5
Glycine	20	17–18
Alanine	13	11
Valine	12	12
Methionine	6	4– 5
Isoleucine	7	6
Leucine	12	15–16
Tyrosine	4	4
Phenylalanine	5	3– 4
Lysine	10	10
Histidine	2	2
Arginine	4– 5	5

Analyses were performed on samples hydrolysed for 24 h, 48 h and 72 h, using a Durham amino acid analyser. Integral numbers for the amino acid residues are calculated from 15 600 for the molecular weight of profilin. Cysteine and tryptophan were not determined

properties: the details of the assay will be published elsewhere (in preparation). Application of this method to extracts of platelets made under polymerising conditions indicate that 45–70% total actin is unpolymerised, even at total actin conc. ~ 5 mg/ml: in general, older batches of platelets contain a lower proportion of unpolymerised actin. From batches of cells which gave 60% and 70% unpolymerised actin, extracts were made in conditions where actin depolymerises (5 mM K_2HPO_4, pH 7.6, 0.1 mM $CaCl_2$, 0.1 mM ATP, 0.2 mM DTE); subsequent addition of 150 mM NaCl and 2 mM $MgCl_2$ resulted in the polymerisation of no more than 40% and 30% actin, respectively. This supports the conclusion that the remaining fraction is polymerisation-resistant.

To test whether this fraction represents profilactin, platelets were sonicated in 1 vol. 5 mM K_2HPO_4, pH 7.6, 150 mM NaCl, 0.1 mM ATP, 0.2 mM DTE. The resulting high concentrations of salt and protein together prevent significant depolymerisation of filamentous actin, although later experiments showed that conditions were not optimal for preservation of actin filaments (in preparation). Centrifugation for 3.5 h at 100 000 × *g* removed polymerised actin, while leaving more than 90% of the DNAaseI-inhibitor activity in the supernatant. After chromatography of the supernatant on Sephadex G-100 in 5 mM K_2HPO_4, pH 7.6, 150 mM NaCl, 0.1 mM $CaCl_2$, 0.2 mM DTE, 80% of the applied DNAase I inhibitor activity was recovered in the peak eluting in the position of profilactin. Electrophoretic analysis of the fractions in this peak and densitometry of the stained gels showed that the average actin: profilin ratio was 0.94:1 (range 0.81–1.12:1). Thus at least 75% of the polymerisation-resistant actin in the original extract is profilactin. No profilin eluted from the Sephadex column in the position expected for the free protein.

A fraction of platelet actin with unusual polymerisation properties has been reported by other workers. Nearly half the platelet actin isolated under depolymerising conditions was resistant to polymerisation [10]. The existence of profilin in platelets can provide an explanation for this observation. In isolating actin from a crude platelet actomyosin preparation [11], it was observed that about 30% of the material was included in a Sepharose 4B column in 0.5 M KCl. This fraction is of interest because it contained primarily 2 polypeptides of mol. wt 45 000 and 16 000 [11]. The relationship between these results and our observations is not yet established.

Platelets are of particular interest in cell motility studies because of their cytological simplicity and the dramatic changes in morphology and ultrastructure resulting from stimulation [3–5]. In view of the high proportion of actin combined with profilin in extracts of these cells, we regard the stimulation of platelets as one of the most promising model systems for studying the role of profilactin in cell motility.

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