Volume 249, number 1, 31-34 FEB 07131 May 1989

Tissue-specific expression of profilin

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Received 16 March 1989

Expression of profilin and profilin:actin ratios in vertebrates were determined with polyclonal antibodies against profilin and actin. Profilin was detected in a wide variety of bovine tissues and was especially enriched in smooth muscle of bovine, porcine and avian origin. The protein was purified from pig stomach muscle tissue. Smooth muscle profilin was found to be more effective in inhibiting the polymerization of skeletal muscle actin than thymus profilin purified by the same method.

Profilin expression; Profilactin, Profilin antibody; (Smooth muscle)

1. INTRODUCTION

In non-muscle cells, the unpolymerized actin probably exists in a 1:1 complex with profilin. Profilin, a small (15 kDa) basic protein, was first discovered in calf spleen [1], but has subsequently been described in various non-muscle vertebrate tissues [2–8] and also in invertebrate cells [9–11]. Recently, it was shown that profilin interacts with PIP_2 [12] and thus represents a link between the PIP_2 cycle and the microfilament system. When profilin reacts with PIP_2 actin it is released from the profilactin molecule, thus this complex can be regarded as a microfilament precursor [13]. However, the PIP_2 cycle is not restricted to non-muscle cells, but was also described for smooth muscle [14]. Moreover, data obtained from Northern blots indicate that profilin is also transcribed in muscle [15]. Since the distribution of profilin in different tissues may reveal more information on its possible functions, we searched for profilin in a wide variety of tissues, using an antibody raised against the thymus protein. In addition, we purified smooth muscle profilin and compared its binding to muscle actin with that of the thymus form.

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Abbreviations: PIP_2, phosphatidylinositol 4,5-bisphosphate; DTE, dithioerythritol; PMSF, phenylmethylsulfonyl fluoride

2. MATERIALS AND METHODS

2.1. *Tissues*

Bovine and porcine tissue extracts were prepared from material obtained at the slaughterhouse. Chicken tissues and cells were obtained from a freshly killed chicken purchased at a chicken farm.

2.2. *Protein purification*

Profilin was purified from pig stomach smooth muscle using a poly-(L-proline)-affinity column as in [16]. 350 g of muscle dissected from pig stomachs was homogenized in 3 vols of extraction buffer containing 50 mM Tris-Cl (pH 7.6), 50 mM NaCl, 20 mM $MgCl_2$, 5 mM EGTA, 1 mM DTE, 1 mM PMSF, 1 ml/l aprotinin (Trasylol, Bayer). After two centrifugation steps, the crude extract was applied to the affinity column. Washing and elution protocols were according to [17]. Profilin containing fractions eluted with 6 M urea were dialysed against 25 mM Tris-Cl (pH 7.6), 2.1 M urea, 15 mM β-mercaptoethanol and were subsequently applied to a Mono-Q-column (FPLC-system: Pharmacia; control unit: Stephan Laage, Bielefeld), equilibrated in the same buffer. Profilin was found in the flow-through fractions. The purified protein was dialysed against 10 mM imidazole (pH 7.4), 0.1 mM ATP, 0.1 mM $CaCl_2$, 15 mM β-mercaptoethanol. Protein concentration was determined spectrophotometrically, using an extinction coefficient for profilin of 1.2 [18]. Actin was prepared from pig or rabbit skeletal muscle as in [19] with an additional gel filtration step on Sephadex G-150.

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00145793/89/$3.50

2.3. *Antibodies*

A polyclonal antibody against calf thymus profilin was raised in rabbits. For immunoblotting, serum was diluted 1:250. A rabbit antibody raised against chicken gizzard actin was used as crude IgG fraction (30 mg/ml). As seen in Fig.2B, lanes 1 and 7, this antibody reacts less intensively with striated muscle actin than with smooth muscle actin (Fig.2B, lanes 4 and 8).

Electrophoretic and immunochemical procedures as well as viscometry were carried out as described [20].

3. RESULTS AND DISCUSSION

3.1. *Characterisation of the profilin antibody and tissue specific distribution of profilin*

The rabbit serum raised against calf thymus profilin reacted selectively with one band of the M_r of profilin when tested on blots derived from crude thymus extracts. This antibody recognized a profilin-like polypeptide in immunoblots of a wide variety of bovine tissues (see table 1 and fig.1). While the signal from striated muscle was weak, smooth muscle extracts (stomach and uterus) showed prominent bands (fig.1B, lanes 3 and 9). In two tissues, spleen and uterus, a double band was stained by the antibody. For calf spleen profilin this phenomenon was ascribed by Malm et al. [21] to partial degradation, resulting in the loss of 2 C-terminal amino acids. However, our results show that the additional band has a higher apparent M_r. Thus, at present, this finding is unexplained in the case of spleen profilins. In the uterus sample, the antibody revealed low amounts of a faster migrating protein, which may be a degradation product.

Table 1

Immunological characterisation of the antibody against calf thymus profilin as tested on immunoblots

	Bovine	Pig	Chicken
1. Skeletal muscle	(+)	-	-
2. Heart muscle	(+)	(+)	-
3. Stomach/gizzard	+	+	+
4. Uterus	+		
5. Liver	+	(+)	+
6. Thymus (calf)	+		
7. Brain	(+)	-	
8. Kidney	+	+	
9. Spleen	+	+	
10. Pancreas	(+)	+	
11. Lymphatic tissue	+		
12. Brushborder cells			+
13. Erythrocytes		-	
14. Blood plasma		-	
15. Fibroblasts	+		(+)

The cross-reaction of the antibody was classified as follows, +: strong reaction, (+): weak reaction, -: no reaction

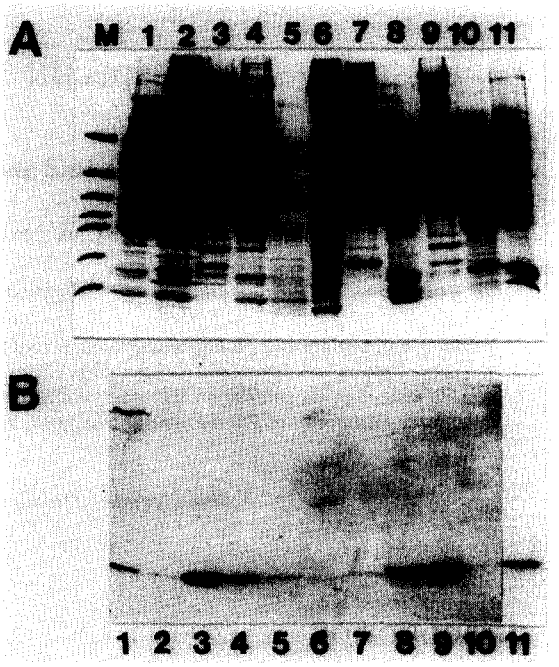

Fig.1. Immunoblot with the antibody against calf thymus profilin and different bovine tissues. A. SDS-gel (8–20%) with different bovine extracts; (M) marker proteins (from top to bottom): 66 kDa (bovine albumin), 45 kDa (egg albumin), 36 kDa (glyceraldehyde-dehydrogenase), 29 kDa (carbonic anhydrase), 24 kDa (trypsinogen), 20.1 kDa (trypsin inhibitor), 14.2 kDa (α-lactalbumin); (1) liver, (2) heart muscle, (3) stomach muscle, (4) kidney, (5), colon, (6) skeletal muscle, (7) brain, (8) spleen, (9) uterus, (10) pancreas, (11) lymphatic tissue. The same amount of probe (based on the fresh weight of tissue) was applied to each slot. B. Immunoblot with anti-profilin from samples identical to A. The gel for blotting contained twice the amount of probe as the gel for staining.

3.2. *Relative amounts of the profilin:actin content in bovine tissues*

To estimate the ratio between the amounts of actin and profilin in a given tissue, parallel immunoblotting experiments were carried out with anti-actin and anti-profilin on several bovine tissues. As expected, the highest concentration of actin was found in muscle tissues. When the same amounts of total protein were applied to the gel (2 mg), profilin could only be detected in spleen, lymphatic

tissue and in smooth muscle extracts (fig.2B), but not in striated muscle.

3.3. *Purification of profilin from pig stomach*

The purification scheme described here (see section 2) is the same as used for calf thymus profilin. This indicates that smooth muscle profilin has chemical and physical properties similar to non-muscle profilin. The different purification steps are shown in fig.3. In contrast to a preparation of non-muscle profilin, the smooth muscle preparation showed large amounts of actin in the 6 M urea fraction eluted from the affinity column (fig.3, lane 8). Thus, smooth muscle actin could not be quantitatively dissociated from profilin by washing with 2 M urea, suggesting, perhaps, that the profilin:actin complex of smooth muscle is more stable. The last urea/anion exchange column [1] yielded profilin preparations more than 94% pure, as judged

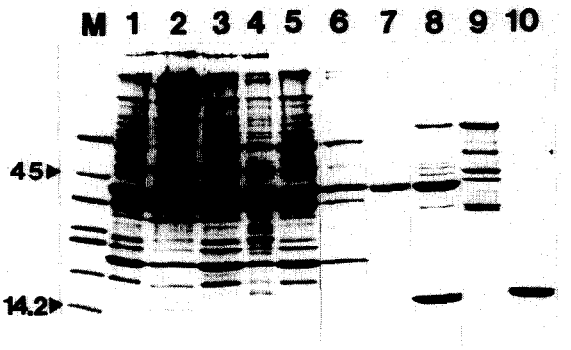

Fig.3. SDS-gel (8–20%) of fractions obtained during the preparation of pig stomach profilin. (M) Marker proteins (see fig.1): (1) supernatant of the crude extract, (2) pellet of the crude extract, (3) supernatant after ultracentrifugation of (1), (4) pellet after ultracentrifugation of (1), (5) flow-through fractions of the affinity column after application of (3), (6) 150 mM wash, (7) 2 M urea wash and (8) 6 M urea eluate of the affinity column, (9) proteins bound to the Mono-Q column after application of (8), (10) purified profilin in the Mono-Q flow-through fractions.

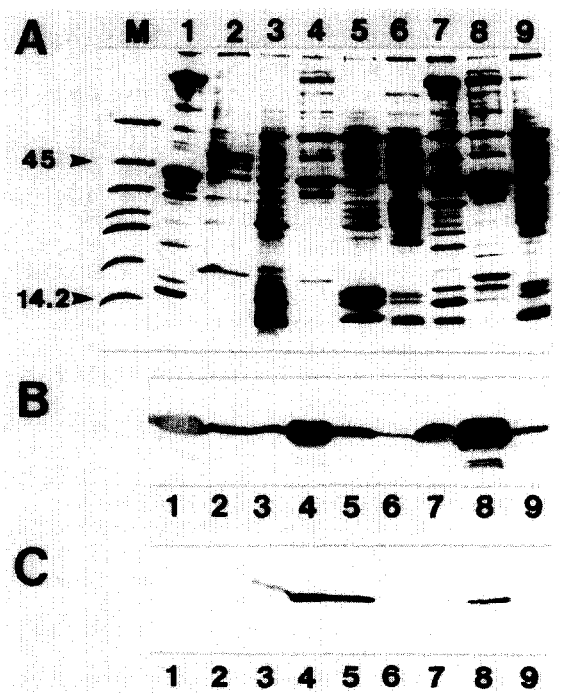

Fig.2 Relative amounts of profilin and actin in different bovine tissue extracts. A. SDS-gel (8–20%) of different bovine extracts, which contained the same amount of total protein (2 mg per slot). (M) Marker proteins (see fig.1): (1) skeletal muscle, (2) brain, (3) spleen, (4) uterus, (5) lymphatic tissue, (6) liver, (7) heart muscle, (8) stomach muscle, (9) kidney. B. Immunoblot with anti-actin IgG (30 mg/ml, diluted 1:200) from samples identical to A, except that each slot contained 4 mg of protein. C. Immunoblot identical to B with anti-profilin serum (1:250).

by densitometry of stained gels (fig.3, lane 10). From 350 g pig stomach smooth muscle, nearly 20 mg profilin could be purified. The actin concentration in smooth muscle was roughly estimated by densitometry to be 15–20% of the total protein. Based on a total protein concentration of 150–200 mg/ml this results in a molar ratio of profilin:actin of approximately 1:200. For human platelets a profilin:actin ratio of 1:10 was described [17]. It is therefore conceivable that smooth muscle contains part of its actin (although less than platelets) as a profilactin complex. However, our data cannot account for the fact that substantial amounts of actin are found in the supernatant derived from smooth muscle homogenates after high speed centrifugation (Hinssen, H. and Small, J.V., personal communication).

3.4. *The effect of pig stomach profilin on actin polymerization*

The influence of profilin on actin polymerization was tested in viscometric assays. In 50 mM KCl without $MgCl_2$, a profilin:actin molar ratio of 0.5:1 prolonged the inital lag phase of actin polymerization by a factor of 2 and reduced the steady-state level to approximately 2/3 of the control value. At a profilin:actin ratio of 1:1, the assembly of actin filaments was completely inhibited (fig.4).

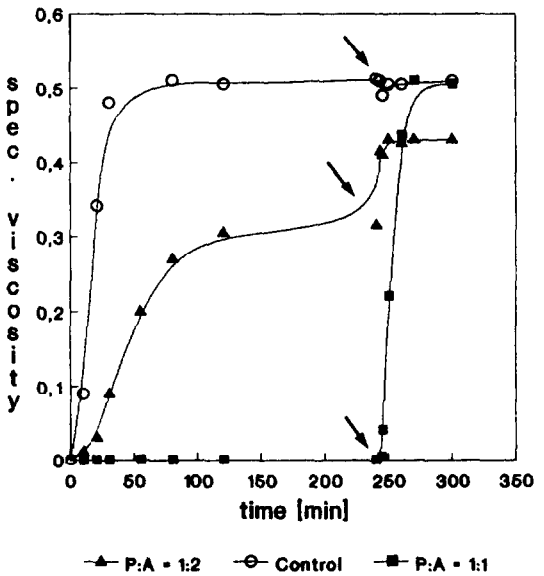

Fig.4. Effect of pig stomach profilin on actin polymerization. After incubation of G-actin (0.5 mg/ml) with various amounts of profilin (1 h at 25°C), polymerization was initiated by addition of 50 mM KCl. As a control, actin was polymerized in the absence of profilin. After steady state had been reached 2 mM $MgCl_2$ was added. (○) Actin control without profilin, (▲) profilin:actin at a molar ratio of 1:2 or (■) 1:1.

The addition of $MgCl_2$ (final concentration 2 mM) dissociated the profilactin complex quantitatively and yielded polymerization-competent actin [18]. For calf thymus profilin quantitative inhibition of actin polymerization could only be observed at a molar ratio of 2:1 (not shown). Thus, smooth muscle profilin had a more profound effect on the polymerization of skeletal muscle actin, as compared with calf thymus profilin purified by the same method. The data for smooth muscle profilin and skeletal muscle actin are similar to those described for the homologous complex between spleen profilin and spleen actin [17]. Further investigation is in progress to analyse possible differences in affinity of smooth muscle profilin for smooth muscle actin or skeletal muscle actin.

Acknowledgements: We thank C. Wiegand and Dr. H. Hinssen for technical advice and helpful discussions. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 223).

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