Expression of scinderin, an actin filament-severing protein, in different tissues

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Scinderin is a calcium-dependent actin filament-severing protein recently discovered in the chromaffin cells of adrenal medulla. In view of the wide tissue distribution of gelsolin, another actin filament-severing protein, experiments were performed to determine the tissue expression of scinderin. Extracts prepared from different bovine tissues were tested by actin-DNase I Sepharose 4B-binding procedure and immunoprecipitation followed by immunoblotting with scinderin and gelsolin antibodies. Among the tissues tested, scinderin was found to be present in the adrenal medulla, brain, anterior and posterior pituitaries, kidney, salivary gland and testis. Scinderin was not found in liver, plasma, skeletal and heart muscles. Gelsolin was expressed in all of the above tissues. The results suggest that scinderin seems to be restricted to tissues with high secretory activity.

Scinderin; Actin; Gelsolin; Actin-binding protein

1. INTRODUCTION

Gelsolin is an actin filament-severing and capping protein found in many tissues [1,2]. It was first identified in rabbit lung macrophages [3] and subsequently found in many mammalian cells [4] including chromaffin cells [5,6]. Brevin is a slightly larger form of gelsolin present in plasma and containing a 25 amino acid extension at its NH_2-terminus [7]. The liver is the main source of this circulating gelsolin.

In our earlier studies on the isolation and characterization of chromaffin cell gelsolin, we observed that another protein was eluted by EGTA-containing buffers together with gelsolin from the DNase I affinity columns [5,6]. This protein was later found to be another calcium-dependent actin filament-severing protein [8,9]. The name of scinderin has been proposed for this protein and studies on its isolation and characterization have been published from our laboratory [8,9]. In view of the wide tissue distribution of gelsolin, studies were carried out to determine the tissue expression of scinderin.

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Abbreviations: SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; Tris-HCl, tris(hydroxymethyl)methylamine-HCl; DTT, dithiothreitol; EGTA, ethyleneglycol-bis-(β-aminoethylether)--*N,N,N',N'*-tetraacetic acid; PMSF, phenylmethylsulfonyl fluoride; ATP, adenosine triphosphate DNase, desoxyribonuclease

2. MATERIALS AND METHODS

2.1. *Preparation of tissue extracts and actin-DNase I Sepharose 4B procedure*

In order to test the tissue distribution pattern of scinderin and gelsolin, a partial purification procedure was used. Twenty-five grams each of the following bovine tissues – adrenal medulla, brain, heart muscle, skeletal muscle, kidney, liver and 100 ml of plasma – were tested. Except for the plasma, the rest of the tissues were washed in ice-cold Locke's solution [10] to remove the blood and homogenised in the same homogenisation buffer used for scinderin purification [9] with the omission of 300 mM KCl (1 g tissue in 4 ml solution), using a Sorval omnimixer. The homogenates were centrifuged at 1000 × *g* for 10 min. The resultant supernatants and the plasma were then centrifuged at 100000 × *g* for 60 min and dialysed against 20 mM imidazole (pH 7.5), 1 mM DTT, 0.25 mM PMSF and 1 mM $CaCl_2$. After the dialysis step $CaCl_2$ and Na-ATP were added to obtain a final concentration of 2 mM and 0.5 mM, respectively. DNase I coupled to Sepharose 4B beads were prepared as described by Bader et al. [6] and saturated with G-actin. The gels were pre-equilibrated with 20 mM imidazole (pH 7.5), 1 mM DTT, 0.25 mM PMSF, 1 mM $CaCl_2$ and 0.5 mM Na-ATP. Supernatants obtained above were mixed with DNase I Sepharose 4B gel and incubated under rotation overnight at 4°C. Each DNase I Sepharose 4B gel batch used had an actin-binding capacity equal to 600 nmol of actin. The preparations were centrifuged at 5000 × *g* for 20 min, the gel sediments were washed several times with the same buffer containing 0.6 M NaCl. Each time the same centrifugation forces were used to sediment the gel. The calcium-dependent actin-binding proteins were eluted by washing the gels with 20 mM imidazole (pH 7.5), 0.25 mM PMSF, 10 mM EGTA, 1 mM DTT and 0.5 mM Na-ATP. Samples from the eluents were used for immunoblotting.

2.2. *Preparation of scinderin*

Scinderin was purified from the adrenal medulla following a four-step chromatography procedure previously described [9].

2.3. *Source of antibodies*

Polyclonal antibodies against gelsolin and scinderin were raised in

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rabbits against purified antigens as described previously [6,9]. Scinderin and gelsolin antibodies have been previously characterized [9].

2.4. *Electrophoresis*

Polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Doucet and Trifaró [11].

2.5. *Immunoprecipitation*

Three grams of each tissue to be tested were homogenized in 5 ml of buffer A (mM: Tris-HCl 20 (pH 7.5), KCl 300, $MgCl_2$ 500, DTT 0.1; soybean trypsin inhibitor (10 μg/ml) and leupeptin (1 μg/ml)) and centrifuged at 5000 × *g* for 30 min. The supernatants thus obtained were further centrifuged at 100000 × *g* for 60 min. Three ml of the supernatants were then incubated for 60 min with 240 μl of a protein A Sepharose CL-4B (Sigma) suspension that had been incubated previously with scinderin antibody for 30 min (1 ml of 5% protein A in buffer B plus 3 ml of 1:20 dilution in buffer B of scinderin antiserum). After this incubation period, samples were centrifuged at 5000 × *g* for 10 min and the pellets thus obtained were washed 3 times with buffer B (mM: Tris-HCl 20 (pH 7.5), EGTA 0.1, KCl 20, DTT 0.1; soybean trypsin inhibitor (10 μg/ml) and leupeptin (1 μg/ml)). The sediments were then boiled in 150 μl of buffer C (mM: Tris-HCl 70 (pH 8.0), EGTA 1, 3% SDS, 10% glycerol, 10% β-mercaptoethanol) for 5 min and then centrifuged at 5000 × *g* for 10 min. The supernatants were boiled in electrophoresis sample buffer for 2 min and electrophoresis and immunoblotting were performed as described above. The immunoprecipitation technique used in the experiments allowed the determination of at least 5 pmol of scinderin per sample with a recovery of 80%.

2.6. *Immunoblotting procedures*

The protocol for immunoblotting was based on the technique of Towbin et al. [12]. Protein samples were processed for immunoblotting as described previously [13,14].

3. RESULTS

Early experiments from our laboratory demonstrated that scinderin was too diluted to be detected by immunoblotting in cytosolic fractions [9]. Consequently, it was necessary to obtain partially purified preparations of scinderin from the tissues prior to testing. The antibodies used in these studies were raised against bovine adrenal medullary scinderin and they did not cross-react with rat adrenal medullary scinderin. Consequently, only bovine tissues were used in the experiments. Four different experiments were performed for each of the following tissues: adrenal medulla, brain, kidney, anterior and posterior pituitaries, skeletal and heart muscles, liver and plasma. Adrenal medulla, a tissue known to contain both gelsolin and scinderin [5,6,8,9] was used as control. Immunoblotting tests performed on the EGTA eluents from the actin DNase I Sepharose 4B gel demonstrated that in addition to adrenal medullary tissue, scinderin was expressed in anterior and posterior pituitaries, brain and kidney, with this latter tissue containing less scinderin than the others (Fig. 1). Immunoblotting tests for scinderin were negative for skeletal and heart muscles and for liver and plasma. This was not due to a low capacity of the affinity gel since (i) up to 600 nmol of actin-binding proteins could be retained per gel and (ii)

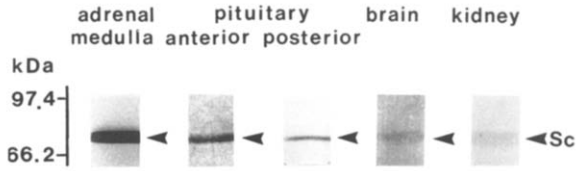

Fig. 1. Tissue expression of scinderin. Samples (150 μg protein) of concentrated EGTA eluents obtained as described in section 2 were subjected to 10% SDS-PAGE and transferred to nitrocellulose membranes. Bovine adrenal medullary extract was used as control. Bovine anterior and posterior pituitary glands, brain and kidneys were tested and found to displace positive immunoreactivity when tested against scinderin antibodies at antibody dilution of 1:500. The position of scinderin in the immunoblots is indicated by arrowheads.

the concentration of scinderin in the adrenal medulla was about 1 nmol/25 g tissue. All samples used in these studies were prepared from 25 g of each tested tissue. Moreover, other actin-binding proteins were found in the EGTA eluents obtained from tissues devoid of scinderin and in order to discard the possibility that these proteins have blocked the binding or adsorption of scinderin on the affinity gels, the following experiment was performed. Cytosol preparations for liver, heart and skeletal muscles and 100 ml plasma were mix-

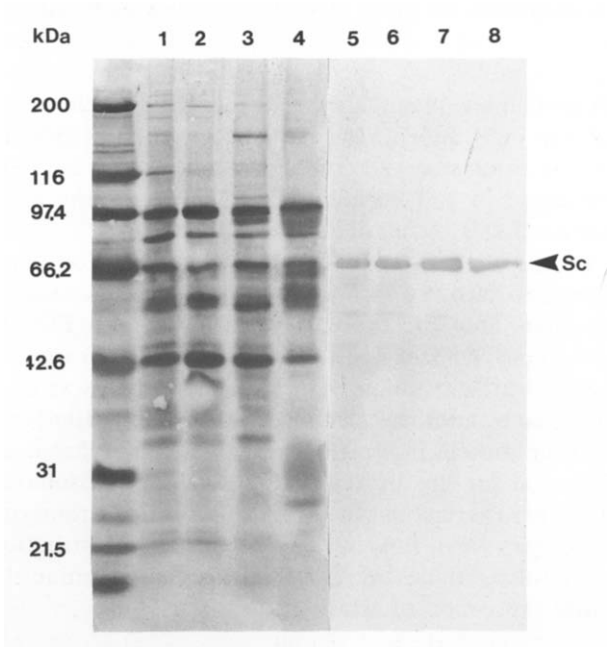

Fig. 2. Lack of interference of actin-binding proteins on scindering adsorption on DNase I Sepharose 4B gels. 25 g of heart (lanes 1 and 5) and skeletal (lanes 2 and 6) muscles, liver (lanes 3 and 7) and 100 ml of plasma (lanes 4 and 8) were processed as described in section 2. 6 ml (2 g) of adrenal medullary cytosol were added to each of the above-described preparations and this was followed by incubation with DNase I Sepharose 4B gels overnight. EGTA eluents (150 μg protein each) were subjected to SDS-PAGE, transferred to nitrocellulose membranes and stained with amido black 10B (lanes 1–4) or incubated with scinderin antibodies (1:500 dilution, lanes 5–8). First lane (St) shows molecular weight standards. An arrowhead indicates the position of scinderin (Sc).

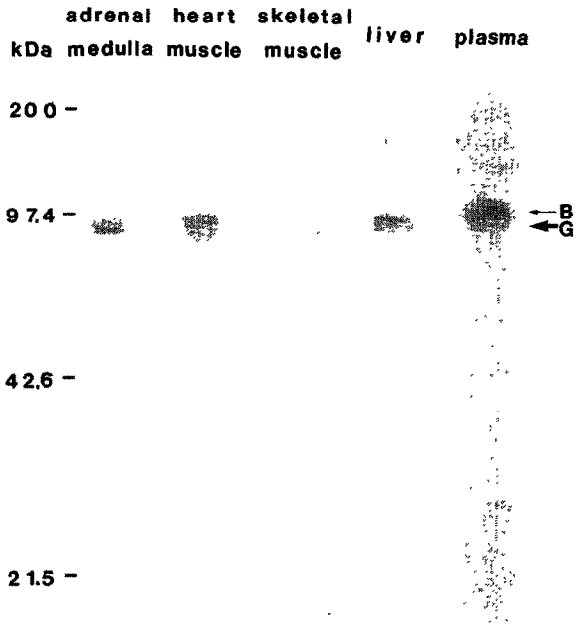

Fig. 3. Presence of gelsolin and/or brevin in adrenal medullary tissue and tissues (skeletal and heart muscles, liver and plasma) which do not contain scinderin. Samples (150 μg protein) of EGTA eluents obtained under similar conditions to those described in legend to Fig. 1, were subjected to SDS-PAGE and then transferred to nitrocellulose membranes and tested with gelsolin antibody (1:1000 dilution). The positions of gelsolin (G) and brevin (B) are indicated by arrows.

ed with 6 ml (2 g) of adrenal medullary cytosol and subsequently added to the DNase I Sepharose 4B gels. EGTA eluents from DNase I Sepharose 4B gels were subjected to SDS-PAGE followed by transfer to nitrocellulose membranes and immunoblotting with scinderin antibodies. In every case, adrenal medullary scinderin was detected in the EGTA eluents (Fig. 2), thus indicating that there was no interference with the binding of scinderin to the affinity gel by other actin-binding proteins present in the preparations. In one of the experiments, transferred proteins were incubated with gelsolin antibodies, in addition to anti-scinderin. Fig. 3 shows that all tissues (skeletal and heart muscles, liver and plasma) that did not express scinderin, did express gelsolin. Moreover, liver and plasma also expressed brevin (Fig. 3). In order to confirm the above results another technique was also used. Immunoprecipitation studies were then performed on cytosolic samples prepared from bovine tissues. The antibody precipitated scinderin from those tissues found to be positive in the above experiments and, in addition, tests on salivary gland and testis also demonstrated the presence of scinderin in these tissues (Fig. 4A). Again in these immunoprecipitation studies, liver, plasma as well as skeletal and heart muscles were found to be negative (Fig. 4A). Experiments in which scinderin aliquots (0.5, 5, 50 and 500 pmol) were added to liver samples demonstrated that this actin filament-severin

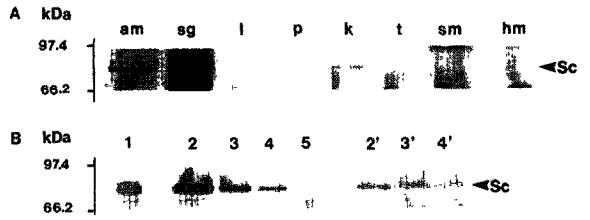

Fig. 4. (A) Immunoprecipitation tests performed with scinderin antibody. The immunoprecipitations studies were carried out as described in section 2. The antibody did not precipitate any proteins from skeletal (sm) and heart muscles (hm) as well as liver (l) and plasma (p) preparations whereas scinderin was found in immunoprecipitates from adrenal medulla (am), salivary gland (sg), kidney (k) and testis (t). (B) In order to test the sensitivity of the method, decreasing amounts of scinderin (pmol: lanes 2 and 2', 500; lanes 3 and 3', 50; lanes 4 and 4', 5; and lane 5, 0.5) were subjected to the immunoprecipitation procedure. Scinderin was incubated alone (lanes 2–5) or in the presence of 3 ml of liver cytosol (30 mg protein; lanes 2'–4'). Lane 1 corresponds to 50 pmol of scinderin applied directly to the SDS-gel.

protein could be precipitated in the presence of liver cytosolic proteins (Fig. 4B). The technique of immunoprecipitation used in the experiments can easily detect 5 pmols scinderin (Fig. 4B); the recovery of scinderin was 80% in the absence and 60% in the presence of liver cytosolic proteins.

4. DISCUSSION

Early work from our laboratory demonstrated the presence in chromaffin cells of gelsolin and scinderin, two Ca^{2+}-activated actin filament-severing proteins [5,6,8,9]. Our studies also demonstrated that scinderin is a structurally different protein from gelsolin [8,9]. Scinderin and gelsolin have different molecular weights, isoelectric points, amino acid composition and yield different peptide maps after limited proteolytic digestion. Moreover, scinderin antibodies do not cross-react with gelsolin and gelsolin antibodies fail to recognize scinderin [8,9].

The present experiments using two different techniques (actin-DNase I-Sepharose 4B procedure and immunoprecipitation) showed that scinderin was strongly expressed in addition to adrenal medulla in brain, salivary glands, anterior and posterior pituitaries. Renal tissue and testis, although found positive were found to contain a much reduced amount of scinderin. The results also showed that scinderin is absent from skeletal and heart muscles, liver and plasma, tissues known to contain gelsolin. Since scinderin was not detected in these tissues and the sensitivity of the immunoprecipitation method used was of 5 pmol, it can be concluded that skeletal and heart muscles, liver and plasma do not contain any scinderin or, if they do, their content is less than 5 pmol/25 g tissue. This concentration of scinderin would be 200 times lower than that present in the adrenal medulla (1000 pmol/25 g). The

present results showing different tissue expressions for gelsolin and scinderin add another fact to the list of differences between these two actin filament-severing proteins. Consequently, the tissue localization of scinderin is more restricted than that of gelsolin. This was not unexpected since villin, another actin filament-severing protein of the gelsolin family, is restricted to tissues whose main function is reabsorption, such as intestine, kidney, biliary and pancreatic ducts [15,16]. Scinderin seems to be expressed in neuronal and endocrine tissues; systems in which secretion is a main function. Early work from this laboratory also demonstrated that scinderin is localized in the subplasmalemmal area of the chromaffin cell [9]. This is the same area that has been shown to contain actin [17,18]. Moreover, work from our [17,19] as well as from other laboratories [18] have indicated that this network of actin is disrupted during stimulation, thus removing a barrier to the movement of secretory vesicles to releasing sites on the inner surface of the plasma membrane. Because of the localization of scinderin in the subplasmalemmal region of the chromaffin cell and of its activation by Ca^{2+}, one is tempted to speculate that cell stimulation and Ca^{2+} entry activate actin filament severing proteins such as scinderin with a consequent breakdown of the actin subplasmalemmal network. This attractive hypothesis is now being tested in our laboratory.

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