Qualitative and quantitative distribution of plasminogen activators in organs from healthy adult mice

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Received 12 October 1985

Twenty organs from healthy adult mice were tested for plasminogen activator activity. All were positive although specific activities varied 200-fold. Tissues with high activity were lung, uterus, brain and kidney. Endocrine glands were moderately rich in activator activity, and lymphoid tissues were poor. Molecular mass characterization was carried out. Two enzymatic forms were observed in all twenty organs: a 70 kDa form similar to human tissue plasminogen activator and a 48 kDa form analogous to mouse urokinase.

Plasminogen activator *Electrophoresis* *(Mouse organ)*

1. INTRODUCTION

Plasminogen activators (PA) are highly specific proteinases which convert inactive plasminogen into active plasmin, a broad spectrum proteolytic enzyme. In humans, two types of PA are found: the tissue-type plasminogen activator (tPA) primarily purified from uterus [1] and the urokinase type (uPA) present in abundance in urine [2]. Both types are detected in many other tissues and fluids. Tumor cells frequently overproduce one or the other type of PA. These two proteins are immunologically distinct, differ in molecular mass, and are encoded by different genes [3,4].

Although numerous data concerning human PA are available, there are only a few reports dealing with PAs of other mammals. Our interest was focused on mouse. In this animal, PA has been observed in a viral transformed fibroblast cell line [5] and in a few embryonic and adult tissues [6,7]. The objective of this study was to quantify the PA activity in healthy adult organs. To identifv the enzymes implicated we used the technique of gel electrophoresis with in situ zymography. Molecular mass characterization could be done for each tissue, even with quantities equivalent to 50 pg PA.

We dedicate this paper to Professor S.P. Datta for his contribution to Biochemistry, his devotion to FEBS and his fundamental role in the success of FEBS Letters

2. MATERIALS AND METHODS

2.1. *Tissue extracts*

3-month-old BALB/c mice were killed by cervical dislocation. Organs were dissected out aseptically and freed of connective tissue and fat. They were then rinsed in a large volume of cold PBS (Dulbecco's phosphate-buffered saline without calcium or magnesium) to eliminate blood contamination. Organs or fragments were gently dried on tissue paper and weighed. They were then minced with scissors and homogenized in 10 vols/wt of cold sterile PBS containing 0.5% Triton X-100 using a Dounce homogenizer (25 strokes of a loose-fitting pestle at 4°C). The lysates were centrifuged for 15 min at $3000 \times g$, and the supernatants stored at -20°C.

2.2. *PA assay*

10 μl homogenate (or an appropriate dilution) were assayed for plasminogen-dependent fibrino-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

lytic activity using ^{125}I-fibrin coated Linbro (24-well) tissue culture dishes. Bovine fibrinogen was purifed according to Mosesson [8] and radiolabelled as described by Goldfine [9] (spec.act. 10^7 cpm/mg). Prior to use, the fibrinogen-coated dishes were incubated for 2 h with thrombin (0.03 U/ml) in PBS at 37°C to convert fibrinogen to fibrin, and then washed twice with cold sterile PBS. Solubilization of ^{125}I-fibrin by tissue extracts in the presence of 8 μg/ml human plasminogen was determined. The plasminogen was purified by lysine-Sepharose chromatography [10]. Results are expressed in terms of equivalent urokinase activity by comparison with a urokinase standard (Choay, France). Total radioactivity was determined by incubating fibrin-coated wells with trypsin (25 μg/ml). Solubilization of radioactivity was linear with urokinase concentration up to 3 mU per assay in a 3½ h incubation. Background release of radioactivity by plasminogen was 2–5% of total radioactivity, and 1 mU urokinase solubilized an additional 15% of the radiolabelled fibrin. Radioactivity released in duplicate assays varied by 10% or less.

2.3. *Gel electrophoresis and zymography of PA*

Samples were electrophoresed under non-reducing conditions in 11% SDS-polyacrylamide slab gels supplemented with plasminogen (20 μg/ml) and gelatin (2 mg/ml) at 4°C [11]. After electrophoresis, the gels were soaked in 1.25% Triton X-100 for 3 h and further incubated in 0.1 M Tris-HCl, pH 8.1, at 37°C overnight. Finally, they were fixed and stained.

3. RESULTS AND DISCUSSION

3.1. *Quantification of PA activity in organs of healthy adult mice*

Twenty organs were tested for PA activity using the ^{125}I-labelled fibrin plate method. The results are summarized in table 1. All organs were positive although with various levels of activity. Specific activities varied 200-fold with the high 'producer organs' being lung, uterus, brain and kidney. However, one must consider that the apparent level of PA is, in fact, the result of several interactions. Specific inhibitors of PAs are sometimes present [12] and may decrease the observed activity. From another point of view, PA may be synthesized as an inactive proenzyme, especially the 48 kDa form [13], and will not be taken into account in this assay. Consequently, the results observed represent a minimal estimation of PA activity in the organ tested. The great abundance of PA(s) in brain was rather unexpected since these enzymes have been frequently associated with cell migration or tissue remodeling. PAs were previously observed in embryonic or neonatal brain but this phenomenon was attributed to neuronal migration [14, 15]. To verify and extend our observation, the mouse central nervous system was dissected in 4 parts: cerebral cortex, cerebellum, diencephalon plus brain stem, and pituitary gland. PA assays showed great differences (table 1) with diencephalon plus brain stem being 7-times richer than cerebellum. Cerebral cortex and pituitary gland were intermediate. These results clearly show that PA is an important enzymatic activity of adult healthy brain although we cannot actually point out which cell type is responsible for this activity.

A group of moderately rich tissues was found which have activities 10–20-times less than that observed in lung. All 4 endocrine glands tested fell in this group: adrenal, testis, thyroid and ovary. These results may be compared to those of Virji et al. [16] demonstrating the presence of PA in the islets of Langerhans. The authors suggested that the PA/plasmin system might play a part in the conversion of proinsulin to the active hormone. In the endocrine glands tested here, 3 out of 4 secrete steroid hormones or adrenergic compounds. PAs have been shown to be involved in both ovulation [17] and spermatogenesis [18]. Their role in adrenal and thyroid remains unknown and it is possible that they could be involved in the endocrine secretion process itself. Connective tissue was also a moderate producer of PA. Concerning muscle, a great difference (6-fold) was observed between heart and skeletal muscle (table 1).

Finally, organs that are considered to be poor producers contained PA at levels 40–200-times below that of lung. Among these are liver, pancreas, and all lymphoid tissues tested: thymus, spleen and lymph nodes. A transformed T cell line (BW) was totally negative. These results are in apparent contradiction with numerous publications dealing with the secrtion of PA by stimulated macrophages [19,20], polymorphonuclear leucocytes [21], B lymphocytes [22], and natural killer

Table 1

Distribution of plasminogen activators in murine tissues

Mouse organs	PA activity (U/mg)	Molecular forms					
		70 kDa		48 kDa		Others	
Lung	2.190	+		+		110 kDa	(traces)
Uterus	1.390	+	(maj)	+		110 kDa	(traces)
Diencephalon + cerebral stem	1.380	+	(maj)	+	(traces)		
Cerebrum	0.623	+	(maj)	+	(traces)		
Kidney	0.531	+		+	(maj)		
Pituitary gland	0.487	+	(maj)	+	(traces)		
Adrenal gland	0.276	+		+			
Testis	0.265	+		+			
Cerebellum	0.191	+	(maj)	+	(traces)		
Thyroid	0.184	+		+			
Heart	0.150	+		+			
Connective tissue	0.130	+		+			
Ovary	0.102	+	(doublet)	+			
Pancreas	0.057	+		+			
Liver	0.046	+		+			
Oesophagus	0.035	+		+			
Thymus	0.032	+	(doublet)	+			
Spleen	0.027	+		+	(maj)	110 kDa	(traces)
Skeletal muscle	0.027	+		+			
Lymph node	0.022	+		+		110 kDa	(traces)
Bladder	0.023	+	(doublet)	+			
Aorta	0.019	+		+		110 kDa	(traces)
Ureter	0.011	+		+		110 kDa	(traces)
Urine (U/ml)	8.250			+			

Tissues were removed, weighed and lysed in PBS containing 0.5% Triton X-100. Lysates or dilutions were assayed by the ^{125}I-labelled fibrin plate method for 3½ h in the presence of plasminogen. Control incubations were also done in the absence of plasminogen to detect possible non-specific proteases. All organs tested were negative for such contamination at the dilutions selected except pancreas and thyroid in which radioactive release represented 3.2 and 7.6% of total solubilization, respectively. These values were subtracted to evaluate PA activity. Molecular masses were characterized as described in fig.1.

lymphocytes [23]. Three possible explanations may be suggested for this difference: (i) unstimulated lymphoid cells may spontaneously secrete low levels of PA; (ii) they may synthesize specific inhibitors [24,25]; and/or (iii) the cultured cells may give divergent results from their corresponding normal tissues due to the culture conditions [26,27].

3.2. *Characterization of PA produced in different tissues*

Two forms of mouse PA have previously been distinguished on the basis of their apparent molecular mass: a 48 kDa PA purified from MSV infected 3T3 cells [5] which is analogous to mouse urokinase and a higher-molecular-mass activator of 70–80 kDa observed in embryonic tissues [28]. The latter can be inhibited by antibodies raised against human melanoma PA. Our aim was to search for other possible PA(s) and to determine the distribution of the already known types of PA.

The activity of these enzymes can still be assayed after SDS-polyacrylamide gel electrophoresis as long as non-reducing conditions are maintained

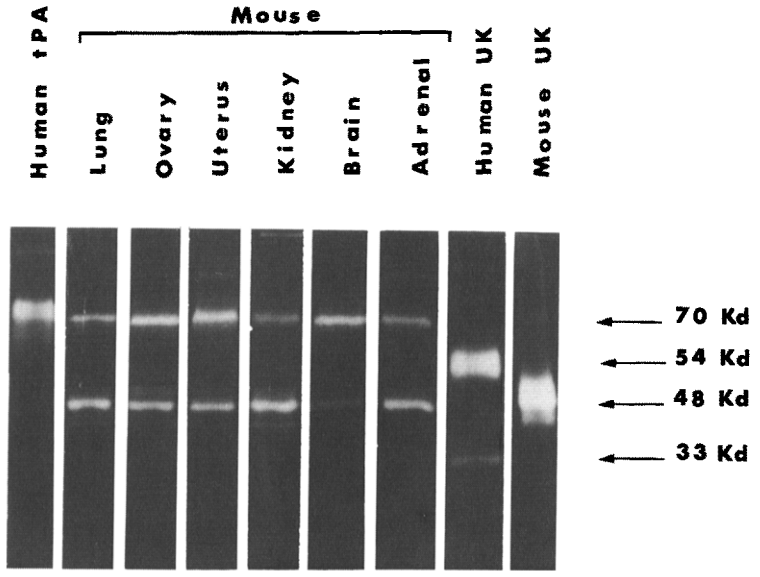

Fig.1. Zymography of electrophoretic gels. Samples corresponding to 5 mU plasminogen activators and adjusted to 1% SDS (without any reducing agents) were loaded on 0.7 mm SDS-polyacrylamide slab gels supplemented with plasminogen and gelatin. Migration was performed at 4°C. Gels were rinsed in 1.25% Triton X-100 and further incubated at 37°C in 0.1 M Tris, pH 8.1. They were finally stained with Coomassie blue. Parallel gels were run in each case without plasminogen to detect non-specific proteases. One such protease is visible in kidney.

during the electrophoresis. The 20 organs were therefore analyzed using these conditions and typical results are shown fig.1 and table 1. All of the tissues exhibited 2 types of PA: the 48 kDa form and a higher-molecular-mass form migrating to the same position as the lighter form of the human tPA doublet (70 kDa). The proportion between the 2 types varied from one tissue to another. At the 2 extremes, kidney contained a majority of the 48 kDa form while brain contained a very large predominance of the 70 kDa form ($\geq 90\%$ of the activity as tested by inhibition by increasing amounts of anti-human tPA immunoglobulins). Occasionally, trace amounts of a 100–110 kDa form were observed in several tissues. Similar high-molecular-mass forms have been previously observed in human cells and demonstrated to be a complex of PA with a specific inhibitor [29].

In conclusion, the proteolytic cascade of PA/plasmin seems to be widely used in organs of healthy adult mice. As in humans, only 2 types of PAs are apparent: mouse tPA showing an identical molecular mass to that of human tPA (70 kDa), and mouse urokinase (48 kDa) with a different molecular mass from human uPA. Both types are observed in all the organs tested although in variable proportion. Of particular interest is the presence of large amounts of mouse tPA in adult brain and to a lesser extent of both forms in endocrine glands.

ACKNOWLEDGEMENTS

We thank Susan Elsevier and Lucile Wiellie-Giroud for their helpful suggestions concerning the manuscript.

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