

# Cathepsin A activity of a parietal thrombus of an abdominal aortic aneurysm

Marta Siergiejuk, Marek Gacko, Anna Worowska

Department of Vascular Surgery and Transplantology, Medical University of Białystok, Poland

**Abstract:** We evaluated the cathepsin A activity of a parietal thrombus of an abdominal aortic aneurysm. We compared this activity to that of a retracted blood clot homogenate. Cathepsin A of aneurysm parietal thrombus homogenate and blood clot homogenate showed the highest activity on Z-Phe-Ala. It was lower on Z-Phe-Phe, Z-Glu-Tyr, Z-Glu-Phe, Z-Gly-Phe, and the lowest activity was on Z-Gly-Ala. We conclude that cathepsin A's activity on a parietal thrombus of an aneurysm is much higher than blood clot cathepsin A activity. (*Folia Histochemica et Cytobiologica* 2011; Vol. 49, No. 1, pp. 10–12)

**Key words:** parietal thrombus of abdominal aortic aneurysm, blood clots, cathepsin A

## Introduction

An aneurysm comprises a widening of the arterial lumen by at least 50%. Aneurysms are caused by atherosclerosis and inflammatory changes as well as enzymatic and immunological processes occurring in the arterial wall [1–3].

Aneurysmal widening of the abdominal aorta is most frequently filled with thrombotic parietal thrombi, the formation of which brings about vascular endothelium damage and disturbs laminar blood flow [4, 5]. It is followed by the adhesion, activation and aggregation of blood platelets, and the platelet plug is strengthened with fibrin fibers [6, 7]. Neutrophils, monocytes, lymphocytes, fibroblasts, and endothelial cells penetrate the aneurysmal thrombus [8, 9]. The thrombus rebuilding and its influence on the aneurysmal wall depend mainly on the activity of the proteolytic enzymes that are present [5, 10].

The aim of our study was to evaluate the specificity and activity of cathepsin A (EC 3.4.16.1) of the parietal thrombus of an abdominal aortic aneurysm. We compared this activity to that of a retracted blood clot.

## Material and methods

Parietal thrombi were collected from 15 patients (14 men and one woman, aged  $65 \pm 5$  years) operated on for aortic aneurysm in the Department of Vascular Surgery and Transplantology of the Medical University of Białystok. The aneurysms were localized below renal branching-off. Retracted blood clots were obtained from the same patients before the operations. The thrombi and the blood clots were stored at  $-75^{\circ}\text{C}$  until they were examined. The whole 10% homogenate of thrombi and blood clots was prepared in 0.15 mol/l of KCl using a knife homogenizer, type Politron, and filtrated through polyamide fabric [11]. Z-Phe-Ala, Z-Phe-Phe, Z-Glu-Tyr, Z-Glu-Phe, Z-Gly-Phe, and Z-Gly-Ala, (Sigma-Aldrich, USA), were used to determine cathepsin A activity in homogenates [12, 13]. Sodium-acetic acid buffer in the amount of 0.1 ml 0.2 mol/l was added to 0.3 ml of homogenate and, after adding 0.1 ml 30 mmol/l of the substrate dissolved in dimethyl sulfoxide, it was incubated at  $37^{\circ}\text{C}$  for four hours. Both homogenate and buffer had a pH of 5.0. The reaction was interrupted by adding 0.5 ml 10% trichloroacetic acid. The samples precipitated at time zero were the controls. The amount of nmols of released C-end amino acid was determined in supernatant fluid obtained through centrifuging, using the ninhydrinic method [14]. The results were expressed as tissue grams and incubation time, as well as protein mg and DNA  $\mu\text{m}$ .

**Correspondence address:** M. Siergiejuk, Dept. of Vascular Surgery and Transplantology, Medical University of Białystok, M. Skłodowskiej-Curie Str. 24a, 15–276 Białystok, Poland; tel.: (+ 48 85) 746 82 77; fax: (+ 48 85) 746 88 96; e-mail: [martasiergiejuk1983@o2.pl](mailto:martasiergiejuk1983@o2.pl)

**Table 1.** Cathepsin A activity of a parietal thrombus of an abdominal aortic aneurysm and blood clots determined on Z-dipeptides (at pH 5.0)

Substrates	C-terminal amino acid [nmol/g/h]		Statistical significance
	Parietal thrombus	Blood clot	
Z-Phe-Ala	18.2 ± 1.72	8.2 ± 0.63	p < 0.001
Z-Phe-Phe	6.4 ± 0.54	2.7 ± 0.26	p = 0.05
Z-Glu-Tyr	2.3 ± 0.19	1.2 ± 0.11	p = 0.058
Z-Glu-Phe	1.6 ± 0.14	1.1 ± 0.09	p = 0.049
Z-Gly-Phe	1.4 ± 0.12	1.0 ± 0.08	p = 0.032
Z-Gly-Ala	0.6 ± 0.07	0.4 ± 0.05	p = 0.45

**Table 2.** Protein and DNA contents and cathepsin A activity of a parietal thrombus and a blood clot expressed as protein milligrams and DNA micrograms

Determination	Parietal thrombus	Blood clot	Statistical significance
Protein, mg/g tissue	38.2 ± 2.92	58.6 ± 5.65	p < 0.05
DNA, µg/g tissue	125.5 ± 13.84	52.6 ± 6.32	p < 0.001
Cathepsin A*, Ala nmol/mg protein	0.476 ± 0.06	0.140 ± 0.01	p = 0.029
Cathepsin A*, Ala nmol/µg DNA	0.145 ± 0.02	0.156 ± 0.02	p = 0.26

\*Substrate: Z-Phe-Ala

The Bradford method was used to determine protein content [15] and DNA content was determined using the Burton method [16].

The results were statistically analyzed using a U Mann-Whitney test, assuming p < 0.05 as statistically significant.

## Results and discussion

Cathepsin A of an aneurysm parietal thrombus homogenate and a blood clot homogenate, expressed as tissue gram and one hour of incubation, showed the highest activity on hydrophobic-hydrophilic substrate Z-Phe-Ala (Table 1). The amounts were 18.2 Ala nmol/g/h for the thrombus, and 8.2 Ala nmol/g/h for the blood clot. The effect on hydrophobic-hydrophobic substrate Z-Phe-Phe was lower. Next was the effect on hydrophilic-hydrophobic substrate Z-Glu-Tyr, Z-Glu-Phe, Z-Gly-Phe. The lowest effect was on hydrophilic-hydrophilic substrate Z-Gly-Ala. The activity of cathepsin A of an aneurysm parietal thrombus homogenate on examined substrates was approximately 1.5 to 2.2 times higher than the activity of cathepsin A on blood clot homogenate. The differences were statistically significant in most substrates. In the case of Z-Glu-Tyr, they were at the borderline of statistical significance. In the case of Z-Gly-Ala they were statistically insignificant.

Protein content in thrombus homogenate was 38.2 mg/g of tissue, while in blood clot homogenate it was 58.6 mg/g of tissue (Table 2). Cathepsin A activity, determined using Z-Phe-Ala expressed as protein, was higher in thrombus homogenate (0.476 Ala nmol/protein mg) than in blood clot homogenate (0.140 Ala nmol/protein mg).

DNA content in the thrombus was 125.5 µg/tissue g. In the blood clot, it was 52.6 µg/tissue g. Cathepsin A activity, determined using Z-Phe-Ala expressed as DNA content, was slightly lower in the thrombus (0.145 Ala nmol/µg DNA) than in the blood clot (0.156 Ala nmol/µg DNA).

On the other hand, expressed as protein content, it was higher in the thrombus homogenate (0.476 Ala nmol/g protein) than in the blood clot homogenate (0.140 Ala nmol/g protein). These differences, apart from the last one, were statistically significant. They resulted from large amounts of blood platelets that contain cathepsin A and do not contain DNA in the thrombus, and large amounts of erythrocytes that do not contain either cathepsin A or DNA in the blood clot [17–19].

Cathepsin A is a lysosomal carboxypeptidase. It splits C-end amino-acids from peptides and proteins, especially quickly if phenylalanine residue occurs in the penultimate position, in a weakly acidic environment [12, 19–21]. Our results are in accord with this.

Cathepsin A participates in protein degradation and activates/inactivates peptic hormones and biologically active peptides [20, 22]. This enzyme has no endogenous inhibitors [23] and its peptidizing activity depends on the availability of the substrate, and a pH of between 4.5 and 5.5 [20, 23]. In a pH of 7.0–7.5, cathepsin A shows amidizing and esterizing activity [22, 24]. It also plays a role as a protective protein to certain glycosidases [25, 26]. These activities of cathepsin A may also apply to the parietal thrombus of an aortic aneurysm.

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