Abstract

The enzyme Disintegrin and Metalloprotease 17 (ADAM 17) is a transmembrane multidomain protease that participates in the processes of cytokine release from the cell surface. This enzyme is involved in the hydrolysis of many protein substrates, so any changes in its activity can lead to disturbances in enzymatic homeostasis, resulting in pathological changes in the body. Many studies show that ADAM 17 is, among other things, associated with the pathogenesis of diabetic nephropathy.

Diabetic nephropathy (DN), also known as diabetic kidney disease, is a serious complication of diabetes. Diagnosing this disease is very complex and currently relies on testing for the presence of protein in urine (microalbuminuria). Unfortunately, this symptom appears only in the third stage of the disease, when changes in the structure of the glomeruli become irreversible. Additionally, elevated protein levels are characteristic of many other kidney diseases. The exact mechanism behind the development of this disease is still not fully understood. Studies show a correlation between the development of DN and increased activity of specific proteases, such as extracellular matrix metalloproteinases and enzymes from the ADAM family, including ADAM 17.

ADAM 17 activity can be monitored using fluorogenic substrates. In this study, the substrate sequence was selected using combinatorial chemistry methods (partitioning and linking). For this purpose, I synthesized two peptide libraries with the general formulas:

$$ABZ\text{-}Asn\text{-}X_3\text{-}X_2\text{-}Ala\text{-}ANB\text{-}NH_2$$

Where: *X_3, X_2 – protein amino acid residues except cysteine*

ABZ – 2-aminobenzoic acid (fluorescence donor)

ANB-NH_2 – amide of 5-amino-2-nitrobenzoic acid (fluorescence acceptor)

$$ABZ\text{-}Asn\text{-}Tyr\text{-}Met\text{-}Ala\text{-}X_1'\text{-}X_2'\text{-}X_3'\text{-}Tyr(3\text{-}NO_2)\text{-}NH_2$$

Where: X_3', X_2', X_1' – protein amino acid residues except cysteine

Tyr(3-NO_2)-NH_2 – amide of 3-nitro-L-tyrosine (fluorescence acceptor)

The deconvolution against ADAM 17 was performed using an iterative method. As a result, a compound with the sequence was obtained:

ABZ-Asn-Tyr-Met-Ala-Leu-Arg-Arg-Tyr(3-NO_2)-NH_2

In the later stages of the study, I analyzed the effect of modifications at the *C*-terminal end of the substrate on the rate of its hydrolysis. Based on the kinetic parameters, the sequence of the most efficiently hydrolyzed substrate was determined:

ABZ-Asn-Tyr-Met-Ala-Leu-Arg-Arg-Lys(DNP)-NH_2

Where: Lys(DNP)-NH_2 – amide of N-ε-2,4-dinitrophenylolysine (fluorescence acceptor)

In this work, I also conducted an analysis of the selectivity of the obtained substrate towards several proteases and demonstrated that the substrate shows high selectivity for ADAM 17. Additionally, proteolytic activity measurements of ADAM 17 enzyme in biological material were carried out. Based on these measurements, I determined the activity of the studied enzyme in the extracellular medium and human podocyte lysates. Furthermore, studies were conducted on urine samples from rats with induced diabetes, where a significant increase in the enzyme's activity was observed. It was also shown that in the urine of diabetic patients, increased activity of this enzyme is observed.