

LIMITED PROTEOLYSIS OF FIBRINOGEN α C-REGION REVEALS ITS STRUCTURE

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α C-regions of fibrinogen (A392-610) are distant C-terminal parts of A α -chains. Their three-dimensional structure is not clear because of high lability [1]. α C-regions cannot be crystallized properly that persuade scientists to search alternative ways of its structure analysis. It is known that proteolytic sites are located in the fragments of polypeptides that have no stable structure. So protease would rather cleave peptide bonds located in unstructured portions of polypeptide and bonds located between parts with distinct structure. That is why limited proteolysis of polypeptides allows to predict peculiarities of protein structure and to verify the three-dimensional computer models [2].

Aim. The purpose of our study was to compare hydrolytic action of proteases from *Gloydius halys halys*, *Agkistrodon contortrix contortrix* and *Calloselasma rhodostoma rhodostoma* snake venoms and from *Bacillus thuringiensis* var. *israelensis* IMV B-7465 culture medium on α C-regions of fibrinogen molecule.

Methods. Products of hydrolysis were characterized by SDS-PAGE under reducing conditions with following Western-Blot using the mouse monoclonal 1-5A (anti-A α 509-610) and II-5C (anti-A α 20-78) antibody. MALDI-TOF analysis of fibrinogen hydrolysis products was performed using a Voyager-DE.

Results. Combination of SDS-PAGE, FPLC and MALDI-TOF analysis allowed us to detect the peptide bonds cleaved by studied proteases. In particular proteases from *Gloydius halys halys* and *Agkistrodon contortrix contortrix* snake venoms cleaved peptide bond A α 413-414. Action of protease from *Calloselasma rhodostoma rhodostoma* on fibrinogen led to the formation of hydrolytic product generated from C-terminal portion of A α -chain that corresponded to fragments generated by enzymes from two other snakes. On the other hand protease from *Bacillus thuringiensis* var. *israelensis* IMV B-7465 culture medium cleaved peptide bond A α 504-505.

Discussion. The specificity of three unlike proteases from different species of snakes towards one particular peptide bond (A α 413-414) indicates that this fragment of α C-regions of fibrinogen

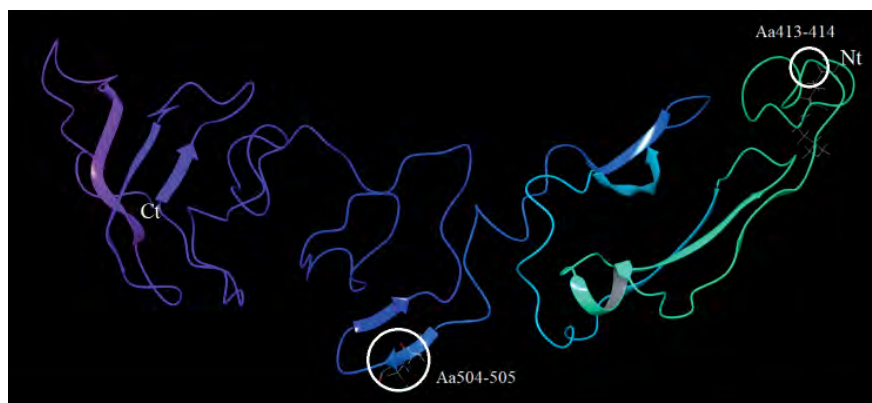


Fig. Three-dimensional structure of fibrinogen α C-region with marked proteolytic sites. Nt — amino-terminus; Ct — carboxy terminus part.

molecule most likely has no secondary structure. Also we can predict that fragments located on both sides of the polypeptide chain from A α 504-505 peptide bond must be exposed for the proteolytic action. These facts have to be acknowledged during 3D-modeling of α C-regions.

It is also notable to say that preliminary data indicate the presence of hydrolytic sites within 414-504 fragment of A α -chain of fibrinogen. In particular such specificity was reported for fibrinogenase from *Echis multisquamatis* venom [3] and fibrinogen-specific protease from *Brahypelma smithi* venom [4]. This fact allows us to conclude the presence of proteolytic site that also can be exposed to the protease or located within a loop of polypeptide chain. Determination of accurate specificity of these proteases will allow to clarify the location of such unstructured sites of the α C-regions. Most likely such unstructured sites are surrounded by parts of molecule that have distinct structure.

Conclusions. Use of limited proteolysis technique as the source of additional information for computer modeling allowed us to propose an improved model of 3D-structure of fibrinogen α C-regions (Fig. 1). This model takes into account the behavior of α C-regions in the physiological condition and contributes to the general knowledge about fibrinogen structure.

Key words: α C-region, fibrinogen, C-terminal, proteases.

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