UDC 577.29 https://doi.org/10.15407/biotech15.02.060

# LIMITED PROTEOLYSIS OF FIBRINOGEN αC-REGION REVEALS ITS STRUCTURE

*Y. Kucheriavyi, O. Hrabovskyi, A. V. Rebriev, Y. Stohnii*

Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv

*E-mail: biophysicist@gmail.com*

Received 25.03.2022
Revized 07.04.2022
Accepted 29.04.2022

αC-regions of fibrinogen (A392-610) are distant C-terminal parts of Aα-chains. Their three-dimensional structure is not clear because of high lability [1]. αC-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* вар. *israelensis* IMV B-7465 culture medium on αC-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-Aα509-610) and II-5C (anti-Aα20-78) 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 Aα413-414. Action of protease from *Calloselasma rhodostoma rhodostoma* on fibrinogen led to the formation of hydrolytic product generated from C-terminal portion of Aα-chain that corresponded to fragments generated by enzymes from two other snakes. On the other hand protease from *Bacillus thuringiensis* вар. *israelensis* IMV B-7465 culture medium cleaved peptide bond Aα504-505.

*Discussion.* The specificity of three unlike proteases from different species of snakes towards one particular peptide bond (Aα413-414) indicates that this fragment of αC-regions of fibrinogen

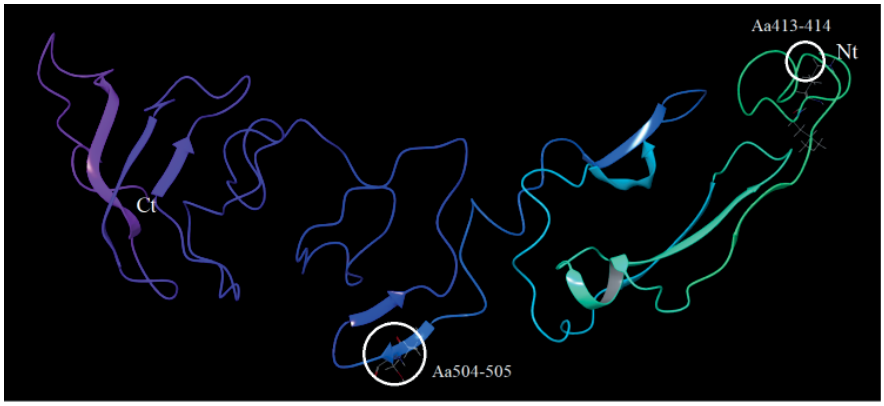


*Fig.* **Three-dimensional structure of fibrinogen αC-region with marked proteolytic sites.**
Nt — amino-terminus; Ct — carboxy terminus part.

molecule most likely has no secondary structure. Also we can predict that fragments located on both sides of the polypeptide chain from Aα504-505 peptide bond must be exposed for the proteolytic action. These facts have to be acknowledged during 3D-modeling of αC-regions.

It is also notable to say that preliminary data indicate the presence of hydrolytic sites within 414-504 fragment of Aα-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 αC-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 αC-regions (Fig. 1). This model takes into account the behavior of αC-regions in the physiological condition and contributes to the general knowledge about fibrinogen structure.

***Key words:*** αC-region, fibrinogen, C-terminal, proteases.

*Acknowledgement*. Authors gratefully acknowledged Dr Chernyshenko for his encouragement of these studies.

*Funding source*. This work was financed by the Palladin Institute of biochemistry of the National Academy of Sciences of Ukraine. PhD-program.

## REFERENCES

1. *Medved L, Weisel J. W*. Fibrinogen and Factor XIII Subcommittee of Scientific Standardization Committee of International Society on Thrombosis and Haemostasis. *Recommendations for nomenclature on fibrinogen and fibrin*. 2009 7(2):355–9. Epub 2008 Nov 25. PMID: 19036059; PMCID: PMC3307547. https://doi.org/10.1111/j.1538-7836.2008.03242.x.
2. *Chernyshenko V. O., Volynets G. P*. Predicting of fibrinogen BβN-domain conformation by computer modeling and limited proteolysis. *Ukr. Bioorg. Acta*. 2011, 1, 53–57. https://doi.org/10.15407/ubj92.03.022
3. *Chernyshenko V. O*. Limited Proteolysis of Fibrinogen by Fibrinogenase from Echis multisquamatis Venom. *The Protein Journal*. 2015, 34(2), 103–104. https://doi.org/10.1007/s10930-015-9605-2.
4. *Kucheriavyi Ye. P., Gryshchuk V. I., Rebriev A. V., Platonov O. M., Savchenko K. S*. The mechanism of anticoagulant action of the venom of Brachypelma smithi. *Modern aspects of biochemistry and biotechnology*. 2021. Kyiv. 20–21 May 2021. P. 29. ISBN 978-617-7026-69-2