UDC 577.1

MAPPING OF RESIDUES OF FIBRINOGEN αC-REGION CLEAVED BY PROTEASE FROM THE VENOM OF *AGKISTRODON HALYS HALYS*

P. Y. TSAP, Y. M. STOHNIY, R. Y. MARUNYCH, A. V. REBRIEV, O. P. KOSTYUCHENKO

Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv; e-mail: bio.cherv@gmail.com

Introduction. Fibrin(ogen) αC-region (Aα220-610) is involved in fibrin polymerization, binds platelet GPIIbIIIa receptor, VLDLR of endothelial cells, plasminogen, tPA, α2-antiplasmin, apolipoprotein, etc. Thus preparation of αC-derived polypeptides could be important issue for studying the role of different parts of the region in physiological processes. Being strongly specific to distinct peptide bonds of proteins, proteases are the promising tool for preparation of such fragments that possess the features or preserve the structure of the whole molecule. That is why the aim of our work was to study the action on fibrinogen of the protease purified from the venom of *Agkistrodon halys halys*.

Methods. Hydrolysis products of fibrinogen by protease from the venom of *A. halys halys* were analysed by SDS-PAGE under reducing conditions with further immunoprobing using the mouse monoclonal 1-6B (anti-Aα509-610) and II-5C (anti-Aα20-78) antibody. Polypeptide, generated at initial synthesis was purified using HPLC chromatography (Agilent 1100, USA) on phenyl-functionalized silica gel (250x4.7 mm, 4.3 ml, Dupont Instruments, Corp., USA), filled with ZORBAX SB-Phenyl. Protein was eluted by 2 M (NH4)2SO4 using linear gradient. MALDI-TOF analysis of purified fibrinogen hydrolysis products was performed using a Voyager-DE Pro (Applied Biosystems, USA). Its accurate molecular weight was calculated using Data Explorer 4.0.0.0. For the identification of peptide the trypsinolysis with following MALDI-TOF analysis was performed.

Results and Discussion. SDS-PAGE showed that protease from the venom of A. halys halys cleaved preferentially the Aα-chain of fibrinogen. Western-blot analysis carried out using monoclonal antibodies allowed us to detect the product with apparent molecular weight of 20 kDa that corresponded to the C-terminal part of Aα-chain of fibrinogen molecule. MALDI-TOF analysis of product of initial hydrolysis of fibrinogen by protease allowed detecting that the main peak occurs at 21,12 kDa. According to "Peptide Mass Calculator" this peptide corresponded to fragment Aα414-610 of fibrinogen molecule. This suggestion was confirmed by analyzing the products of tripsinolysis, protein sequence coverage was 94%.

Conclusions. It was shown that protease from the venom of *A. halys halys* cleaves the peptide bond AαK413-L414. Its application allowed us to obtain unique non-physiological product Aα414-610 that represents mainly the C-terminal subdomain of fibrin(ogen) αC-region.

Acknowledgement. Authors gratefully acknowledge Dr E. Lugovskoy and Dr I. Kolesnikova for their help with the production and selection of antibodies used in study.