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ACTION OF VENOM OF VIPERA SNAKE OF UKRAINE ON BLOOD COAGULATION *in vitro*

E. Iskandarov [1, 2], *O. Zinenko*[3], *A. Tupikov*[4], *A. Pitishkina*[1, 2], *O. Platonov*[1], *V. Gryshchuk*[1], *Y. Kucheriavyi*[1], *Y. Stohnii*[1]

[1]Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv
[2]Educational and Scientific Center "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv, Ukraine
[3]V. N. Karazin Kharkiv National University, Ukraine
[4]Society for Conservation GIS

E-mail: iskandarov.e.sh@gmail.com

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Hemorrhagic action caused by phospholipases is the main toxic action of *Vipera* snakes venom [1]. However, action on the blood coagulation system is studied less broadly.

Aim. In this study we focused on the search of fibrinogen-targeted proteases in the venom of *Vipera renardi, Vipera nikolskii* and *Vipera berus*. Venom of *Vipera berus* was also fractionated on Q-sepharose and action of separated fractions on human blood plasma, platelets and red cells was studied.

Methods. Analysis of protein mixtures was performed using SDS-PAGE. Action on the blood coagulation system was analyzed using the APTT assay. Identification of protein components with fibrinolytic activity was performed using enzyme-electrophoresis with fibrinogen as the substrate [2]. Fractionation of *V. berus* venom was performed on Q-sepharose using FPLC system Acta Prime. Action of separated fractions on ADP-induced platelet aggregation in platelet rich blood plasma was analyzed using Aggregometer AP 2110 [3]. Hemolytic action of fractions was estimated using fresh human red cells. Amount of released hemoglobin was estimated by spectrophotometry on Optizen POP.

Results. All studied venoms had different protein compositions with major protein fractions in the range from 25 kDa to 130 kDa. Both *V. berus* and *V. nikolskii* venoms taken in 1:200 dilutions reduced the time of clotting in APTT test from 25 to 13 s. In contrast, *V. renardi* venom in the same dilution prolonged the clotting time from 25 s to 180 s that we assumed as the result of fibrinogen-specific protease presence. According to enzyme-electrophoresis data all studied venoms contained fibrinogen-specific proteases with the apparent molecular weights for *V. berus, V. nikolskii* — 25–55 kDa. and *V. renardi* — 55–75 kDa. Fractionation of crude venom of *V. berus* allowed obtaining several fractions eluted at different concentrations of NaCl: 0.1; 0.2; 0.3; 0.5 M. Non-binded fraction was also collected. The results of analysis of their action on compounds of the blood coagulation system are presented on Table.

Discussion. We assumed that in the case of *V. berus* and *V. nikolskii* the fibrinogenolytic action is masked by procoagulant components. However action on fibrinogen by the components of studied venoms cannot be neglected. Also we have to keep in mind that several purified snake venom proteins have become significant devices in fundamental exploration and in diagnostic procedures in hemostasiology. That is why further studies of fibrinogen-specific proteases of these species' venoms are promising. We also obtained fibrinogen-specific protease from *V. berus* venom. Its action substantially decreased the fibrinogen polymerization and also disrupted the ability of fibrinogen to support platelet aggregation.

Conclusions. Thus, the components of *Vipera* venoms living in Ukraine can be used for basic biochemical research. At the same time, care should be taken in the case of envenomation, as the presence of fibrinogenolytic enzymes in the venom can lead to hemorrhage. Further characterization of fibrinogen-specific protease from *V. berus* venom is a promising task for biotechnology.

Table. **Results of the analysis of fractions' properties of venom of Vipera berus berus. NB is the fraction that did not bind to the Q-sepharose under present conditions**

	Prolongation of the clotting time of plasma	Hemolysis of red cells	Activation of platelets	Inhibition of platelet aggregation
N.B.	+	–	+	–
0.1	–	–	+	–
0.2	+	–	–	+
0.3	–	+	–	–
0.5	–	+	–	–

Note. 0.1; 0.2; 0.3; 0.5 — fractions eluted at a NaCl concentration of 0.1; 0.2; 0.3; 0.5 M.

Key words: snake venom, *Vipera renardi*, *Vipera berus nikolskii*, *Vipera berus berus*, fibrinogenolytic action, fibrinogen-specific protease, APTT, enzyme-electrophoresis.

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