

ACTION OF VENOM OF *Vipera lebetina* ON BLOOD COAGULATION *in vitro*

E. ISKANDAROV^{1,2}, A. DMYTRUK^{1,3}

¹Palladin Institute of biochemistry of the National Academy of Sciences of Ukraine

²Biology And Medicine Institute Science Educational Center
of Taras Shevchenko National University Of Kyiv, Ukraine

³National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute",
Ukraine

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

Received 2023/04/03

Revised 2023/04/11

Accepted 2023/04/28

Hemorrhagic action caused by phospholipases is the main toxic action of *Vipera* snakes' venom [1]. However, action on the proteins of blood coagulation system is also spread widely among these toxins. Proteases with unique specificity are known tools for the studying structure and functions of blood coagulation system proteins. Even venoms with prevalence of hemolytic or neurotoxic action can contain a wide range of proteases that are of interest for biotechnology and drug development.

Aim. In this study we focused on the search of fibrinogen-targeted proteases in the venom of *Vipera lebetina*.

Methods. Fractionation of the venom was performed using FPLC chromatographic system Acta Prime on Q Sepharose. Analysis of protein mixtures was performed using SDS-PAGE. Action on blood coagulation system was analyzed using APTT assay [2]. Proteolytic action on fibrinogen and identification of protein components with fibrinolytic activity was performed using electrophoresis of mixture of fibrinogen solution (2 mg/ml) with venom's fractions. For a comprehensive evaluation of the effect of the obtained fractions on hemostasis, an original approach with modified aggregatometry was used [3]. This approach made it possible to take into account the ability of fractions to activate platelets, initiate blood coagulation, or inhibit platelet aggregation. Hemolytic action of fractions was estimated using fresh human red cells. Amount of released hemoglobin was estimated by spectrophotometry on Optizen POP.

Results. Crude venom of *V. lebetina* was fractionated using ion-exchange chromatography on Q Sepharose. Elution was performed using a stepwise gradient of NaCl (0.1, 0.2, 0.3, 0.5, and 0.7 M NaCl) in 0.05 M Tris-HCl buffer at pH 8.3. Fractions eluted at 0.1 and 0.2 M of NaCl contained several proteins with different molecular weights ranging from 75 kDa to low molecular weight fractions according to the SDS-PAGE. In particular, it was necessary to isolate the major fraction with a molecular weight of approximately 20 kDa in fraction 0.1. In fractions 0.3, 0.5, and 0.7, proteins were present in small concentrations. Proteins that cleave α - and β -chains of fibrinogen were found in fractions 0.1 and 0.2, indicating the presence of an enzyme with fibrinogenolytic activity in the venom of *V. lebetina*. The fractions 0.3, 0.5, and 0.7 did not show any significant fibrinogenolytic activity. After platelet aggregation study we concluded that fraction 0.1 contained a protein with fibrinogenolytic activity. An increase in platelet aggregation was observed for the fraction 0.2 after the addition of ADP. This may indicate the presence of an active compound that promotes platelet aggregation. Further research is necessary to determine its nature. Fractions 0.3, 0.5, and 0.7 had no effect on platelet aggregation. A decrease in blood plasma clotting time in APTT to 5 s and 7 s, compared to a control value of 70 s, was shown for fractions eluted at NaCl concentrations of 0.1 M and 0.2 M, respectively. The fractions 0.3, 0.5 had only a slight effect on reducing blood plasma clotting. A slightly increased level of hemolysis was shown in the presence of the unbound fraction and the whole venom. It can be suggested that proteins with phospholipase activity are present in the non-bound fraction.

Table 1

Results of the complex analysis of *Vipera lebetina* snake venom fractions

	Reduction of the clotting time of plasma	Hemolysis of red cells	Fibrinogen cleavage	Inhibition of platelet aggregation
N.B.*	+	+	+	+
0.1	+	–	+	+
0.2	+	–	+	–
0.3	+	–	–	–
0.5	+	–	–	–
0.7	–	–	–	–

* — NB is the fraction that did not bind to the Q sepharose under present conditions. 0.1, 0.2, 0.3, 0.5, 0.7 — fractions eluted at a NaCl concentration of 0.1, 0.2, 0.3, 0.5, and 0.7 M NaCl M respectively).

Discussion. Thus, during the study we demonstrated the presence of proteins in *V. lebetina* venom with fibrinogenolytic activity and proteins that initiate the plasma coagulation time in the APTT test and may have properties of a thrombin-like enzyme or prothrombin activator. Based on our findings, *V. lebetina* venom can be used as a source for obtaining proteins with thrombin-like or prothrombin activating effects (Table 1). 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 hemostasis study. That is why further studies of fibrinogen-specific proteases of these species' venoms are promising.

Conclusions. Thus, fibrinogen-specific proteases, hemolytic agents, activators of blood clotting were found in the venom of *Vipera lebetina*. Most of these compounds must to be purified and can be used for basic biochemical research.

Keywords: snake venom; *Vipera lebetina*; fibrinogenolytic action; protease, APTT; aggregatometry; hemostasis.

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

Authors' contribution. EI fractionated venom of *V. lebetina* and performed measurements, AD performed data analysis and help in the study.

Funding source. The research was financed by the project 0119U002512: “The interaction of the hemostasis system components at the cellular and molecular level in the process of formation and elimination of a thrombus.”

REFERENCES

1. Fernandes C.A., Borges R.J., Lomonte B., Fontes M.R. A structure-based proposal for a comprehensive myotoxic mechanism of phospholipase A2-like proteins from viperid snake venoms. *Biochim Biophys Acta*. 2014, 1844(12), 2265–2276. <https://doi.org/10.1016/j.bbapap.2014.09.015> Epub 2014 Sep 30. PMID: 25278377.
2. Iskandarov E., Zinenko O., Tupikov A., Pitishkina A., Platonov O., Gryshchuk V., Kucheriavyyi Y., Stohnii Y. Action of venom of *Vipera* snake of Ukraine on blood coagulation *in vitro*. *Biotechnologia Acta*. 2022, 15(2), 56–57. <https://doi.org/10.15407/biotech6.02.097>
3. Iskandarov E., Gryshchuk V., Platonov O., Kucheriavyyi Y., Slominskyi O., Stohnii Y., Vartanov V., Chernyshenko V. Fractionation of *Vipera berus berus* snake venom and detection of bioactive compounds targeted to blood coagulation system, *Southeastern European Medical Journal*. 2022, v. 6, p. 20–31. <https://doi.org/10.26332/seemedj.v6i2.256>