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Sh.A. Topchiyeva *

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Review Article

Comparative Analysis of Total Venom Protein (Macrovipera Lebetina Obtusa Linnaeus, 1758) With Different Shelf Life

Sh.A. Topchiyeva

Institute of Zoology of the Ministry of Science and Education of the Republic of Azerbaijan.

***Correspondence Author:** Sh.A. Topchiyeva, Institute of Zoology of the Ministry of Science and Education of the Republic of Azerbaijan.

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Abstract

Animal toxins are a complex of natural biologically active compounds, but there are gaps in the comprehensive study of snake venoms, including in the study of the venom of the Transcaucasian viper. The purpose of the study is a comparative analysis of the total protein of the venom of viper (Macrovipera lebetina obtusa Linnaeus, 1758) with different storage periods. Protein concentrations were determined using the Bradford method. We conducted experiments to determine the total protein in samples of viper venom that were collected in 1989, 1991, 1993, 2010, 2015. As a result of experimental studies, a decrease in the total protein content of viper venom was revealed during long-term storage in the period from 1989 to 2015 and amounted to 55.0%, 59.8%, 73.6%, 83.4%, 93.0%, respectively. The results of the data obtained can be used in the identification, standardization and determination of the biological activity of both individual biologically active components of the Transcaucasian viper venom and drugs based on them.

Keywords: venom of viper; macrovipera lebetina obtuse; total protein; bradford method

Introduction

Zootoxins are a complex of natural biologically active compounds, which include proteins, lipoproteins, peptones, mucin and mucin-like substances, purine bases, salts, various trace elements, epithelial cell remains, proteolytic enzymes that destroy proteins, protease and esterase enzymes that clot blood, and a number of others. Animal venoms are extremely rich and complex natural sources of biologically active molecules, which are classified according to their origin and their effect on the body [1-3]. Animal toxins cause significant dysfunction in the nervous, cardiovascular and muscular systems. Poisoning by animal poisons causes serious pathological disorders in humans and can lead to death [4-6]. Despite the comprehensive study of snake venoms [7-13, 14, 15], there are gaps in the study of the venom of poisonous animals, including the venom of the Transcaucasian viper. Significant variation in the content of serine proteases and metalloproteases has been revealed in snake venoms. In the venom of the common viper (Vipera berus), 75% of the total proteolytic activity is due to metalloproteases, 25% to serine proteases. In the venom of the viper (Vipera lebetina), metalloproteases account for 15% of the total proteolytic activity, while serine proteases provide 85% of the activity of the venom [16-1, 18, 19]. Neurotoxins and other biologically active substances from the venoms of snakes of the genera Naja and Vipera are promising. a renewable source for the production of new generation pharmaceuticals. When supplying snakes with poison, one should take into account the area of residence of snakes and age characteristics, obtain homogeneous poisonous preparations containing biologically active components in a certain ratio. Modern chromatographic methods ensure the isolation of highly purified peptides and enzyme preparations from snake venoms that have anesthetic, antibacterial, anticancer, immunomodulatory, antihypertensive, anticonvulsant, opioid-like, anti-inflammatory, and wound healing activities. The use of such biological products with a high level of activity will make it possible to create new medicinal formulas with a pronounced therapeutic effect when using either a small dose of active ingredients. Neurotoxins and other bioactive substances of genera Naja and Vipera snake venoms are promising renewable source for production of new generation of pharmaceutical preparations. During snake venom provision it should be taken into account the area of snake residence and age characteristics, to obtain a homogeneous venom preparation, containing biologically active components in a certain ratio. Modern chromatographic techniques provide isolation of highly purified peptides and enzyme preparations from snake venoms with anesthetic, anti-bacterial, anti-cancer, immunomodulatory, anti-hypertensive, anti-convulsive, opioid-like, anti-inflammatory, wound healing activities. The usage of such biological products with high-level activity will give an opportunity to create new medicinal formulas with pronounced therapeutic effect in the use of either small dose of active ingredients [20-21]. Viper's venom contained a significant amount of protein (830-930 μg/mg of venom) and exhibited high proteolytic activity towards tyrosine (80-140 μg/min mg of protein). Proteolytic activity did not depend on the season, as well as the age or physiological state of snakes during the reproductive period [22]. Viper venom was administered at a dose of 50 μg/100 g body weight, with an LD50 of 1.9 mg/kg body weight and a total protein content of 97.8%. The venom of the Caucasian viper (Vipera lebetina obtusa) was obtained by mechanical milking of snakes by one of the authors [23]. From the above literature data, it follows that despite a comprehensive study of the toxic and pharmacological effects of snake venoms on living organisms, the given partly contradictory data on methods of separation, identification, standardization and isolation of venom toxins using physicochemical and biochemical methods, there are a number of unresolved questions and gaps in this aspect.

The purpose of the research is a comparative analysis of the total protein of the venom of the viper (Macrovipera lebetina obtusa Linnaeus, 1758 with different shelf life. The research material was whole venom of the Transcaucasian viper (Macrovipera lebetina obtusa), dried in a desiccator over calcium chloride vapor and samples of venom with different shelf life. The poisonous secretion of the Transcaucasian viper was dried under standard conditions in a desiccator over calcium chloride at room temperature for at least 10-12 days. Next, the crystalline poison was collected and analyzed. Samples of viper venom were stored in glass containers in the refrigerator at a temperature of +5-60 C. With this method of drying and storage, the poison retained its biological activity for at least 3 years.

Research results

Over the last century, various methods have been developed for the quantification of proteins for the determination of total protein content and for single proteins. Common methods for protein quantitation include traditional methods such as UV absorbance at 280 nm, bicinchoninic acid and Bradford assays, as well as alternative methods such as Lowry. Bradford's analytical method is based on the formation of a complex between the dye Coomassie Brilliant Blue G-250 and proteins in solution with arginine and hydrophobic amino acid residues). Free dye exists in four different ionic forms. The more anionic forms bind to proteins and have an optical density at 590 nm. Protein concentration can be estimated by determining the amount of dye in ionic form and measuring the absorbance of the solution at 595 nm using a spectrophotometer. The dye binds mainly to arginine, tryptophan, tyrosine, histidine, and phenylalanine in protein molecules. At protein concentrations ranging from 2 μg/ml to 120 μg/ml, a linear relationship is observed. The Bradford protein concentration method has been successfully used to measure solutions with low protein concentrations and solutions containing components that also have significant absorbance at 280 nm. The Bradford protein concentration method, like the Lowry method, requires the construction of a standard calibration curve. To construct a calibration curve, the ratio of the volume of the reagent to the protein sample under study was taken to be 50:1. To prepare Bradford's working reagent, take 100 mg of Coomassie G-250 (Coomassie Brilliant blue G-250) and dissolve it in 50 ml of 95% alcohol, then add 100 ml of orthophosphoric acid (85%), bring it to 1 liter with distilled water and filter through a paper filter. We prepared a pure Bradford solution, which was brown in color and turned blue in the presence of protein. After bringing the volume to 1 liter with bidistilled water, the brown solution again acquires a bluish tint. After 5-7 days, the solution turns brown again. Bovine serum albumin (BSA) with a concentration of 1 mg/ml was used as a standard. To construct a calibration curve, we placed 0, 2, 5, 10, 25, 50, 100, 250 μg/ml BSA into each cuvette with a micropipette. To do this, 0.002, 0.005, 0.01, 0.03, 0.05 were placed in each cuvette. 0.1, 0.3, 0.5 ml of BSA solution in 0.9% sodium chloride solution. 1 ml of 0.9% sodium chloride solution was placed in the control cuvette. The BSA concentration was determined using a Spekol 1500 spectrophotometer (Analytik Jena, Germany). The volume of the cuvette contents was adjusted to the 1 ml mark with 0.9% sodium chloride solution and 0.5 ml of Bradford reagent was added, followed by mixing. The concentration of viper venom was determined using a calibration curve constructed using bovine serum albumin. Optical density measurements were carried out after 20 minutes at a wavelength λ=595. Experimental data for the spectrometric determination of total protein according to Bradford are presented in Table 1.

№	Amount of protein, μg/ml	Optical density, nm
1	2,0	0.04±0.002
2	5,0	0.08±0.003
3	10,0	0.11±0.012
4	25,0	0.24±0.015
5	50,0	0.44±0.021
6	100,0	0.92±0.015

Table 1: Bradford spectrometric determination of total protein (M±m) n=5

We have obtained a direct proportional dependence of the albumin concentration on optical density in the range from 0 to 100 μg/ml. Statistical processing of experimental data was carried out using Students test. We conducted experiments to determine the total protein in samples of viper venom with different storage periods (venom samples were collected in 1989, 1991, 1993, 2010, 2015).

Year of venom collection	Total protein, %
1989	55.0 ±3,8
1991	59.8±5,5
1993	73.6±9,9
2010	83.4±9.2
2015	93.0±10,8

Table 1: Total protein data in viper venom samples with different shelf life

As can be seen from Table 1, the content of total protein in samples of viper venom over the period of storage since 1989, with extension of the storage period, decreased by 1.69, 1.56, 1.26, 1.12 times compared to the venom sample collected in 2015. In all likelihood, as a result of biochemical changes occurring in samples of snake venom, there is a decrease in enzyme activity from 93.0% to 83.4, 73.6, 59.8, 55.0%, respectively, which must be taken into account during the production and storage of both native venom and preparations based on zootoxin. Thus, from the above it follows that the duration and storage conditions of snake venom have a significant impact on both enzymatic activity and toxicity, and on the content of total protein a of snake venom. With long-term storage of viper venom samples from the storage period of 1989, 1991, 1993, 2010, 2015, a significant decrease in the total protein content is observed and amounts to 55.0%, 59.8%, 73.6%, 83.4%, 93.0%, respectively. The results of experimental data can be used in the identification, standardization, preparation of preparations and determination of the biological activity of both individual biologically active components of the Transcaucasian viper venom and preparations based on them. It should be noted that the wide range of peptides and proteins with different biological functions makes animal venoms a valuable source of new compounds, both for use in basic research and for the development of new drugs.

Conclusions

1. Experimentally revealed a decrease in the total protein content of viper venom during long-term storage in the period from 1989 to 2015 from 93% to 55%.
2. A decrease in the total protein of viper venom was revealed by 1.69, 1.56, 1.26, 1.12 times, respectively, which is associated with biophysical and biochemical processes in venom samples that were stored depending on the period and conditions and storage period of the snake venom.

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