Research Article
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Isolation and identification of anticoagulant components from the venom of honey bee (apis mellifera caucasica)

Abstract

The paper presents experimental data on the separation, identification and isolation of anti-coagulant components of the venom of the honey bee Apis mellifera Caucasica, harvested from apiaries from the ecologically clean zone of Azerbaijan. The protein components of zootoxin with molecular masses 41kD and 20kD, 15kD, corresponding to hyaluronidase and phospholipase was isolated from honey bee venom by the method of gel chromatography on a column with Sephadex G-750 eluting with 0.4M sodium phosphate buffer, followed by spectrophotometric measurement of the unit optical density of the fractions at λ=280nm on a Hitachi-557 spectrophotometer.

Keywords: honey bee, apis mellifera caucasica, anticoagulant venom

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Introduction

Despite the presence of a large arsenal of hormonal drugs, antibiotics and other new potent chemotherapeutic drugs, bee venom remains among the most effective medicines, the use of which is expanding. The mechanism of toxic effect of bee venom is very complex and is the result of a complex effect of its components on various organs and systems. Bee venom increases the amount of hemoglobin and blood leukocytes, reduces its viscosity and coagulability and dilates capillaries and small arteries, increasing the flow of blood to the organs.[1] Separate components of bee venom can be used to achieve certain biological effects. Bee venom also affects the central and peripheral nervous system and can be used to treat patients with heart disease. In the literature, data on the use of bee venom for the treatment of patients with various degenerative diseases of the nervous system, such as multiple sclerosis, Alzheimer's disease and Parkinson's disease and others[2–7] have been published. H. Zolfagharian, M. Mohajeri, M. Babaie revealed that the bee venom increases the clotting time. By the authors, the honey bee venom were divided into fractions by using gel filtration and chromatography on Sephadex G-50 and their molecular weight was determined by using electrophoresis using sodium dodecyl sulfate in a polyacrylamide gel. Column gel chromatography isolated F1 fraction containing hyaluronidase, F2 and F3 containing phospholipase and F4 containing melittin with molecular masses of 3, 15, 20 and 41kDa, respectively. It was noted that fractions F2, F3 and F4 had a greater anticoagulant activity than fraction F1. Thus, the authors consider bee venom as a complex of substances containing an anticoagulant factor consisting of 4 protein fractions with molecular masses of 3, 15, 20, and 41kDa. A lethal dose of the whole LD50 venom was determined to be 177.8μg/mouse.[8] Despite numerous studies on the study of bee venom, the isolation and identification of poison components, a number of questions on the study of their effect on the coagulating blood system of experimental animals are available, the study of which is of great scientific and practical interest. Proceeding from the foregoing, the purpose of these studies was to isolate the anticoagulant fractions from the venom of the honey bee Apis mellifera Caucasica, collected from apiaries from the ecologically clean zone of Azerbaijan.

Material and methods

The material of the study was the whole venom of the honey bee Apis mellifera Cau-casica, collected from bees from apiaries located in the area of the ecologically clean zone of Azerbaijan, from the territory of the Ismail area. After storage, the venom was stored in a desiccator over a couple of calcium chloride. Venom solutions were prepared immediately before the experiment. Separation of the poison into fractions was carried out by column chromatography on a Sephadex G-75 column measuring 15x150mm.

To identify the protein components of bee venom, we developed a model technique for the separation of marker proteins. The molecular weights of the marker proteins were determined on a Sephadex G-75 column. For preparation of the column, the matrix G-75 gel was soaked for 48 hours. The prepared gel suspension was carefully filled into a chromatography column. After the height of the layer of the settled gel reached 5cm, a column crane was opened and a stream of pre-prepared solvent was passed through it, observing the conditions under which the rate of solvent effluent from the column was much less than the flow rate of the solvent during chromatography. After uniform gel settling, the column was washed with a buffer solution and again left for 12 hours at the temperature of chromatography. 0.4M Na-phosphate buffer solution with a pH value of 7.0 was selected as the eluent. The volume of the investigated solution of the venom did not exceed 1 ml. The elution was carried out with a 0.04M Na-phosphate buffer solution at pH 7.0 and at a rate of 8ml/hr.

Research results and discussions

At construction of the calibration curve, the protein-marcers: Cytochrome C with Mm=12kD, trypsin with Mm=20kD, erythrocyte spacecraft with Mm=30kD and albumin lyophilized from human serum with Mm=67kD were used.. Further, a mixture of marker proteins of 5mg was passed through a separating chromatographic glass column. The fractions were collected in separate 4.0ml tubes, followed by measuring the optical density on a spectrophotometer. The quantitative data of spectrophotometric separation of marker proteins are given in (Table 1). Table 1 presents the optical density data

of the marker-protein fractions separated by gel chromatography on a Sephadex G-75 column. Further, the collected fractions, separated by elution with a 0.4M solution of Na-phosphate buffer pH 7.0, were combined into separate solutions of marker proteins, followed by measurement of their optical density (Table 2).

Table 1 Data of spectrophotometric determination of the unit of optical density of protein-marker fractions separated by gel filtration on a column with Sephadex G-75

No. of fractions	The unit of optical density of fractions	No. of fractions	The unit of optical density of fractions	No. of fractions	The unit of optical density of fractions	No. of fractions	The unit of optical density of fractions
1	2	3	4	5	6	7	8
1	0.01	15	0.041	29	0.026	43	0.038
2	0.015	16	0.038	30	0.022	44	0.036
3	0.032	17	0.036	31	0.020	45	0.032
4	0.035	18	0.031	32	0.018	46	0.028
5	0.038	19	0.033	33	0.032	47	0.042
6	0.040	20	0.035	34	0.034	48	0.046
7	0.048	21	0.032	35	0.036	49	0.060
8	0.052	22	0.034	36	0.040	50	0.063
9	0.055	23	0.34	37	0.050	51	0.069
10	0.060	24	0.068	38	0.085	52	0.052
11	0.064	25	0.045	39	0.060	53	0.042
12	0.075	26	0.032	40	0.048	54	0.032
13	0.046	27	0.030	41	0.035	55	0.021
14	0.054	28	0.028	42	0.033	56	0.010

Table 2 The separation of marker proteins by gel filtration on a column with Sephadex G-75

No. of fractions	Protein markers	VR, ml	M, thousand daltons
1	Albumen	48	67
2	KA Erythrocyte	96	30
3	Trypsin	172	20.1
4	Cytochrome C	204	12

As can be seen from these tables, the marker proteins were arranged in descending order of elution volume - VR, corresponding to an increase in the molecular mass of proteins. Based on the data presented in (Figure 1), it can be seen that the direct proportional dependence of the marker proteins is in the range 12-67kD. Thus, on the basis of experimental data, the separation conditions of the marker proteins were determined by column chromatography using Sephadex G75 followed by spectrophotomet-ric determination of molecular weights, the isolated components in the range of 12-67kD. For the separation and identification the proteins of zootoxin, we sampled 10 mg of bee venom, which were dissolved in 1ml of bidistilled water and pipetted onto the Sephadex G-75 surface by means of a pipette. Elution of the bee venom proteins was carried out with 0.04M sodium phosphate buffer. The fractions were collected in a volume of 4ml, followed by a spectrophotometric measure-ment of the unit optical density of the samples at λ=280nm on a Hitachi-557 spectrophoto-meter. The data of chromatographic separation of bee venom proteins by the gel filtration method on a column with Sephadex G-75 are presented in (Tables 3) (Table 4) and in Figure 2.

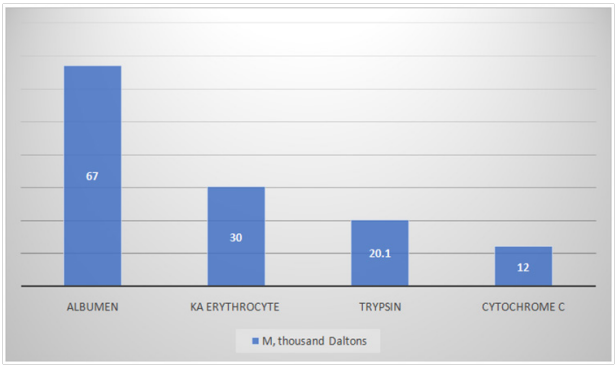

Figure 1 Direct proportional dependence of the marker proteins is in the range 12-67 kD.

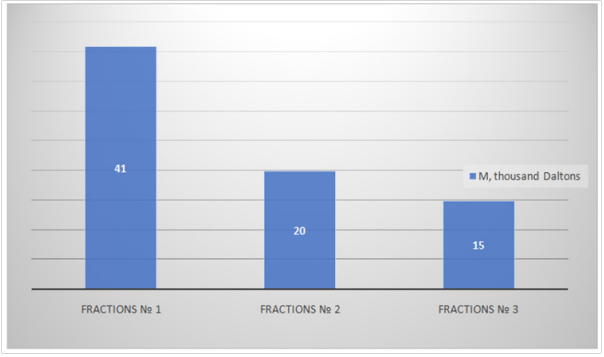

Figure 2 Direct proportional dependence of the bee venom proteins is in the range 15-41 kD.

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Table 3 The optical density data of the honey bee venom fractions separated by gel filtration on a Sephadex G-75 column

No. of fractions	The unit of optical density of fractions	No. of fractions	The unit of optical density of fractions	No. of fractions	The unit of optical density of fractions
1	0.01	22	0.012	43	0.050
2	0.018	23	0.011	44	0.062
3	0.041	24	0.012	45	0.076
4	0.045	25	0.010	46	0.040
5	0.052	26	0.001	47	0.034
6	0.031	27	0.010	48	0.022
7	0. 012	28	0.010	49	0.015
8	0.011	29	0.011	50	0.012
9	0.010	30	0.012	51	0.011
10	0.012	31	0. 012	52	0.010
11	0.001	32	0.011	53	0.010
12	0.010	33	0.010	54	0.010
13	0.010	34	0.012	55	0.010
14	0.011	35	0.025	56	0.010
15	0.010	36	0.036		
16	0.010	37	0.048		
17	0.010	38	0.056		
18	0.010	39	0.078		
19	0.011	40	0.062		
20	0.010	41	0.036		
21	0.010	42	0.022		

Table 4 Data on the separation of honey bee venom by gel filtration on a Sephadex G-75 column

No. of fractions	VR, ml	Mm, kD
1	20.0	41.0
2	156.0	20.0
3	180.0	15.0

Table 3 shows optical density data of the honey bee venom fractions separated by gel filtration on a Sephadex G-75 columnAs can be seen from (Table 4), as a result of bee venom fractionation by gel chromatography on a column with Sephadex G-75, the investigated venom samples were separated into fragments of fractions of 3 proteins with molecular weights from 15 to 41kD. From these tables, it can be seen that the components of the bee venom are arranged in order of increasing elution volumes-VR, which correspond to the decrease in the molecular masses of proteins. Comparing the obtained data with the data of published sources, it can be stated that the isolated components of the bee venom with molecular masses of 41kD correspond to hyaluronidase and 20kD, 15kD to phospholipase. The data of chromatographic separation of by the gel filtration method on a column with Sephadex G-75 are presented in (Tables 3) (Table 4) and in. Thus, by the method of column chromatography elution with 0.04M Na-phosphate buffer, optimal conditions for the fractionation of the venom of the honey bee were determined by gel chromatography on a column with Sephadex G-75.

Acknowledgements

i. Optimal conditions for the separation and identification of proteins of honey bee venom were developed by gel chromatography on a column with Sephadex G-75 eluting with 0.04M Na-phosphate buffer.

ii. Hemocoagulating proteins of hyaluronidase and phospholipase with molecular masses of 41kD and 20kD, 15kD, respectively, were isolated from the honey bee venom.

Conflict of interest

The authors declare there is no conflict of interest.

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