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Protein pattern of the honeybee venoms of Egypt

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ABSTRACT

The venom composition of the Egyptian honeybee *Apis mellifera lamarckii*, the Carniolan honeybee *Apis mellifera carnica* and a hybrid with unknown origin were analyzed using electrophoresis (SDS-PAGE). All venoms shared six bands with molecular weights of 97.400, 67.400, 49.000, 45.000, 43.000 and 14.000D. Bands with molecular weights of 81.000, 56.000, 37.000 and 10.000D are characteristic for the Egyptian subspecies. Bands with molecular weights of 12.000 and 8.000 are characteristic for the Carniolan subspecies, while bands with molecular weights of 32.000, 20.000 and 2.835D are characteristic for hybrid bees. It was noticed that Egyptian bee venom and the hybrid bee venom have strong similarities in their biochemical composition.

KEYWORDS: social insects, toxins, biochemistry, electrophoresis.

INTRODUCTION

Gel electrophoresis of protein is a widely used technique in insect molecular systematics. The technique relies on the fact that identical proteins migrate the same distance under the electrical force applied to an electrophoretic gel, while non-identical proteins usually migrate different distances (Berlocher 1984).

In general, the study of venoms including those of insects, has profoundly affected modern biochemistry, pharmacology, and medicine. These secretions have provided excellent sources of highly concentrated active enzymes, cytotoxins, and neurotoxins to serve as research tools in order to study the subtle subcellular functioning of mammalian nervous and cardiovascular systems. Insect venoms also provide almost unlimited opportunities to investigate the functioning of insect nervous and muscular systems. Insect venoms previously found little direct use in modern medicine, but this situation is now rapidly changing as more information is becoming available. As new techniques for isolating, identifying, and especially for mass producing individual venom components are developed, the above uses and roles of venoms will certainly increase.

The low molecular weight biogenic amines (histamine, dopamine, noradrenaline, etc.) are involved in the local reaction and after a single sting they can lead to systemic reaction. They act on blood vessels and nerve endings, inducing swelling, redness, pain and itching. Major toxic effects of the venom can be attributed to the larger peptides such as melittin, apamin and mast cell degranulating factor. These peptides can damage the cell membranes leading to liberation of enzymes from lysozymes and of mast cell granula, and to cytolysis. Additonally, they can act as neurotoxins, provoking hyperexcitability. The enzymes with a higher molecular weight, with the exception of the highly cytotoxic phospholipase A2, are regarded as less harmful. Hyaluronidase has an indirect effect by increasing the penetration of the active peptides (Meir & White 1995).

The various enzymes and vasoactive components induce a toxic local inflammation in the sting region. If the sting occurs in a highly vascular area or even intravascularly, the toxic components are spread readily and might give rise to systemic reactions. Several simultaneous stings will cause more reactions.

The protein patterns of the venom of the Egyptian honeybee *Apis mellifera lamarckii,* the Carniolan honeybee *Apis mellifera carnica,* and a hybrid with unknown origin have been

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analyzed using polyacrylamide gel electrophoresis. This venom analysis was used to shed light upon the structure and the correlation between venom composition of the commonest honeybee strains in Egyptian fields.

MATERIALS AND METHODS

Venom collection: The pure Egyptian and Carniolan honeybees and the hybrids were collected from the Bee Research Department, Plant Protection Institute, Ministry of Agriculture, Cairo. Pure venom was obtained by the method of Schmidt (1986). Frozen bees were thawed, sting apparatus was removed into a spot of distilled water. The venom reservoir was pinched off, removed from the rest of the sting apparatus and rinsed with distilled water. The venom was squeezed out of the reservoirs. The whole venom was dehydrated over silica gel for 3 days.

Electrophoresis: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli (1970) with 15-20% polyacrylamide concentration. Molecular weight markers (29,000-205,000D) were used as standards. Densitometric scanning of the standard markers was used to show the different concentration of each band (Figure 1). Tentative identification of the substances in venoms was carried out by comparing the gels with those from the existing literature. Proteins were visualized with coomassie blue stain.

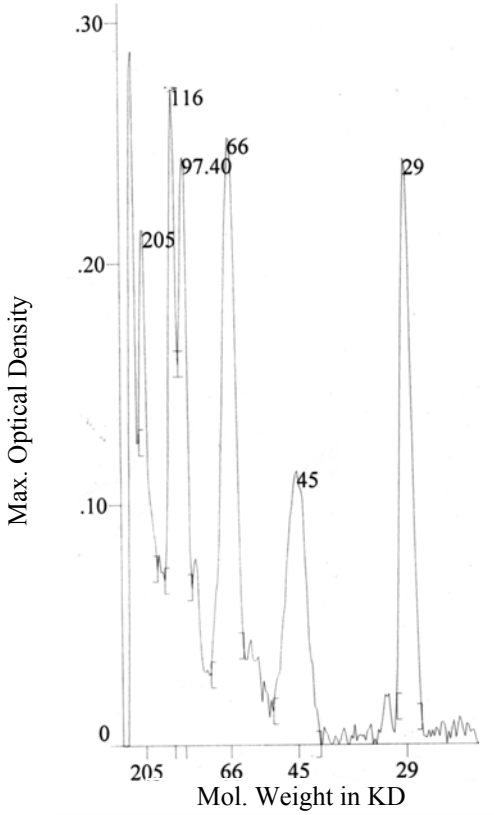

Fig. (1) Densitometric scanning of the standard markers showing the different concentrations of each band.

Data analysis: Similarity of the honeybee strains was measured using Jaccard Index (J) (Ludarig & Reynolds 1988) as following: J = a/a+b+c; where a = bands shared between two races; b = total number of bands in strain 1; c = total number of bands in strain 2.

RESULTS

For the Egyptian honeybee, *A. m. lamarckii,* venom, as shown from figure (2) and table (1), twelve bands were obtained. These bands had molecular weights of 97.400, 81.000, 67.400, 56.000, 49.000, 45.000, 43.000, 37.000, 14.000, 10.000, 6800 and 5.200D. From densitometric scanning (figure 2) it was evident that the band with molecular weight 14.000D has the highest concentration, followed by band number 10 with a molecular weight of 10.000D.

For the Carniolan bee, *A. m. carnica,* venom (Figure 2 and Table 1), nine bands with molecular weights of 97.400, 67.400, 49.000, 45.000, 43.000, 14.000, 12.000, 8.000 and 6.400 D were obtained. Densitometric measurements, show that the band with a molecular weight of 14.000D has the highest concentration, followed by band number 8 with molecular weight 11.869D.

For the hybrid venom (Table 1), thirteen bands were obtained. The bands have molecular weights of 97.400, 67.400, 49.000, 45.000, 43.000, 32.000, 2.000, 14.000, 12.000, 10.000, 6.800, 5.200, and 2.835D. Densitometric measurements show that band number 9 has the highest concentration, with a molecular weight of 14.000D, followed by band number 10, with a molecular weight of 12.000 D, and number 11, with a molecular weight of 10.000D.

The degree of similarity between different honeybee venoms (Table 1) showed that bands with molecular weights of 81.000, 56.000, 37.000 and 10.000D characterize the venom of the Egyptian honeybee. Bands with molecular weights of 6.400 and 8.000D are characteristic for Carniolan bees. While bands with molecular weights of 32.000, 20.000, and 2.835D are characteristic for hybrid bees.

The Egyptian, Carniolan and hybrid bees share six bands with molecular weights 97.400, 67.000, 49.000, 45.000, 43.000 and 14.000D. Egyptian and Carniolan bee venoms are similar in sharing six bands with molecular weights of 97.400, 67.000, 49.000, 45.000, 43.000, and 14.000D. Egyptian and hybrid bees share nine bands with molecular weights of 97.400, 67.000, 49.000, 45.000, 43.000, 41.000,10.000, 6.800 and 5.200D, while Carniolan and hybrid venom share seven bands with molecular weights of 97.400, 67.000, 49,.000, 45.000, 43.000, 14.000 and 12.000D.

Using the Jaccard index equation to measure the similarity index between the honeybee venoms, it was found that the highest degree of similarity occurs between *A. m. lamarckii* and the hybrid (0.26). The degree of similarity between *A. m. carnica* and the hybrid is 0.24. The least similarity occurs between *A. m. lamarckii and A. m. carnica* (0.2).

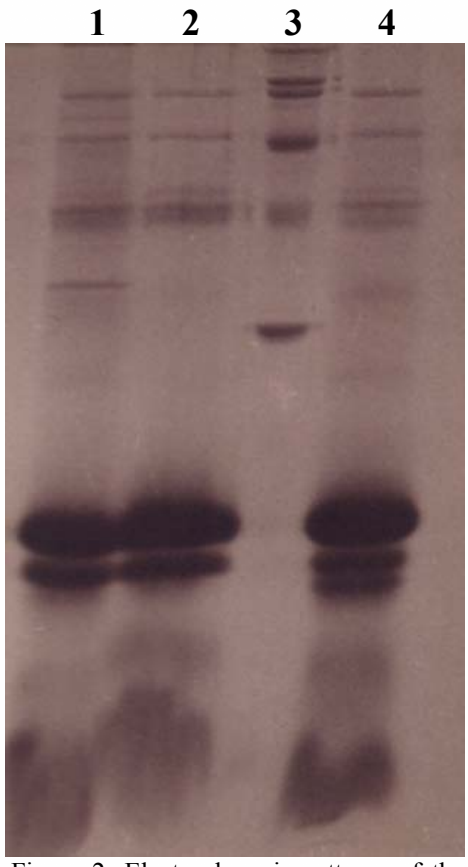

Figure 2: Electrophoresis patterns of the venoms of : 1, *Apis mellifera lamarckii* ; 2, *Apis mellifera carnica* ; 3, Standard markers (from top to bottom: 205.000, 116.000, 97.400, 66.000, 45.000, 29.000D) ; 4, Hybrid

No.	Mol. Wt. (D)	*A. m. lamarckii*	*A. m. carnica*	Hybrid	Comment
1	97.400	+	+	+	Not known
2	81.000	+			Form of acid phosphomonoesterase
3	67.400	+	+	+	Form of acid phosphomonoesterase
4	56.000	+			Hyaluronidase
5	49.000	+	+	+	Hyaluronidase or phospholipase A
6	45.000	+	+	+	Form of phosphatase or hyaluronidase
7	43.000	+	+	+	Form of phosphatase or hyaluronidase
8	37.000	+			Form of hyaluronidase or phospholipase A
9	32.000			+	Phospholipase A or hyaluronidase
10	20.000			+	Phospholipase A or lysophospholipase or antigen 5
11	14.000	+	+	+	Phospholipase A
12	12.000		+	+	Phospholipase A
13	10.000	+		+	Phospholipase A or melittin (tetramer)
14	8.0000		+		Protease or form of phospholipase A
15	6.800	+		+	Protease inhibitor
16	6.400		+		Protease inhibitor
17	5.200	+		+	Minimine
18	2.835			+	Melittin

Table 1: Total number of bands detected using gel electrophoresis for the three honeybee strains. (*A. mellifera lamarckii* "total bands = 12" , *A. mellifera carnica* "total bands = 9" and the hybrid "total bands = 13"). + = band present

DISCUSSION

For *A. m. lamarckii* venom, twelve bands were obtained with molecular weights ranging from 97.400 to 5.200D. The band with molecular weight of 97.400D is of very high molecular weight and no reports about this compound are available. The bands with molecular weights of 81.000 and 67.4000D could be acid phosphomonoesterase. The molecular weights of phosphatase enzyme, as judged by SDS gel electrophoresis, are about 45.000 and 90.000D (Banks & Shipolini 1986) and 49.000D (Schmidt 1982). The bands with molecular weights of 56.000, 49.000, 45.000 and 43.000D could be either form of hyaluronidase. For hyaluronidase enzyme, the molecular weights reported are 50.000 and 35.000D (Banks & Shipolini 1986), 60.000D (Dimitrov & Natchev 1977), 30.000D (Sobotka *et al*. 1976), 37.000D (Kemeny *et al*. 1981) and 53.000D (Hoffman *et al*. 1977). The band with molecular weight 37.000D could be a form of phospholipase A. The molecular weight of one form of phospholipase A enzyme is 38.500D (Banks & Shipolini 1986). The bands with molecular weights of 14.000 and 10.000D could be either form of phospholipase A. For phospholipase enzyme, the molecular weights reported are 14.555D (Banks & Shipolini 1986) and 10.400D (Jentsch & Dielenberg 1972). The band with molecular weight 6.800D might be protease inhibitor. Lowry *et al*. (1971) isolated a peptide, minimine, which has a molecular weight of 6.000D. This peptide could be that found in Egyptian bee venom with molecular weight 5.200D.

The densitometric measurements of the Egyptian subspecies show that the bands with molecular weights of 13.247 and 11.696D could be phospholipase. These bands were detected with the highest peak of density in the Egyptian bee venom.

For *A. m. carnica* venom, nine bands were obtained with molecular weights ranging from 97.400 to 6.400D. The band with molecular weight 67.400D could be acid phosphomonoesterase. The bands with molecular weights of 49.000, 45.000 and 43.000D could be either form of hyaluronidase. The bands with molecular weights of 14.000 and 12.000D could be either form of phospholipase A. The band with molecular weight 8.000D may be phospholipase A or protease inhibitor. The band with molecular weight 6.400D might also be protease inhibitor, although according to Shkenderov (1973) this should produce a band with a molecular weight between 8.000 and 10.000D.

For the Carniolan subspecies the bands with molecular weights of 13.944 and 11.869D could be phospholipase A. For the hybrid venom, thirteen bands were obtained with molecular weights ranging from 97.400 to 2.835D. The band with molecular weight 67.400 D could be acid phosphomonoesterase. The bands with molecular weights of 49.000, 45.000 and 43.000D could be either forms of hyaluronidase. The bands with molecular weights of 32.000 and 20.000D could be forms of phospholipase A. The bands with molecular weights 14.000 and12.000 D could be forms of phospholipase A. The band with molecular weight 6.800D might be protease inhibitor. This peptide could be found in hybrid bee venom as 5.200D. The band with molecular weight 2.835D could be melittin (Habermann 1972; Shipolini 1984; Banks & Shipolini 1986).

Densitometric measurements of the hybrid show that the bands with molecular weights of 13.944, 11.956 and 10.870D could be phospholipase A.. These bands also have the highest concentrations in the Egyptian and Carniolan venom indicating that phospholipase A may have the highest concentration of the venom. An extra compound with molecular weight 10.870D has been detected which is unique to this hybrid strain.

It is also clear that the *A. m. lamarkii* and hybrid venoms are more similar to each other than either is to the *carnica* venom; this may reflect the dominance of genetic characters of the *lamarkii* subspecies over those of *carnica.* This suggests that *lamarkii* genes may be expressed more in the hybrid than are those of the *carnica* bee.

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الملخص العربى

التركيب البروتينى لسم نحل العسل فى مصر

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تم تحليل تركيب السم لثلاثة سلالات من نحل العسل (*آبيس ميلليفيرا لآماركى* "نحل العسل المصرى"، *آبـيس ميلليفـرا كارينكا* "نحل العسل الكارنيولى"، ونحل عسل هجين "غير معروف أصل الأبوين") وذلك باسـتخدام جهـاز الفصـل الكهربائي (الإلكتروفوريسيس) لمعرفة التركيب البروتينى لسم كل نوع وبيان علاقة الأنواع الثلاثة ببعضها من حيـث المكونات الرئيسية لسمومها

تشابه سم السلالات الثلاثة من النحل محل الدراسة فى إحتوائها على ستة مركبات بروتينية، كانت أوزانها الجزيئيـة: 97, 67، 49 ، 45 ، 43 ، 14 كيلو دالتون . بينما تميز سم النحل المصرى عن السلالتين الأخر تـين فـى وجـود مركبات بروتينية ذات اوزان جزيئية 81، 56 ، 37 و 10 كيلو دالتون . وأحتوى سم النحل الكارنيولى على مركبات بروتينية ذات أوزان جزيئية 12 و 8 كيلو دالتون لم يتم تسجيلها فى السلالتين السابقتين. أما سم النحل الهجين فقد تميز بوجود مركبات بروتينية ذات أوزان جزيئية 32، 20، 2 كيلو دالتون . أيضا أوضحت دراسة مدى تقارب الأنواع مـن بعضها بناء على التركيب البروتينى للسم وذلك بإستخدام معامل جاكرد أن تركيب سم النحل المصرى يتشابه بصـورة كبيرة مع سم النحل الهجين مما قد يفسر أن النوع المصرى ربما يكون له الغلبة الوراثية فى التعبير عن تركيبه الوراثى فى الطوائف المهجنة فى مصر أكثر من سلالة النحل الكارنيولى