Application of High-performance Cation-exchange Chromatography for Characterization of Stinging Insect Venoms *

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In recent years clinical and immunological studies have shown that insect venom preparations are reliable for diagnosis and treatment of stinging insect allergy.¹ Venom of common insects such as

bees and vespids are complex mixtures of biogenic amines, peptides and proteins (enzymes) with both pharmacological and allergenic activities.² The use of modern biochemical separation techniques has made it possible to isolate and characterize individual venom components. The methods used for separation and characterization of venoms from stinging insects of the order Hymenoptera are mainly based upon gel chromatography, ion-exchange chromatography and electrophoresis.^{3,4} However, the conventional gel types used for gel filtration and ion-exchange chromatography lack mechanical strength which is a major limitation when operating with high mobile-phase-velocities. In such cases the soft gels cannot be used and there is a requirement of rigid packing materials. We have previously shown that size exclusion chromatography at low pressure on a hydrophilic polyester packing⁵ might be useable for rapid characterization of insect venoms. In this communication we report

* Communication at the Meeting of the Swedish Biochemical Society in Uppsala, 20–21st December, 1982.

Fig. 1. Ion-exchange chromatographic separation of bee venom. Conditions: Mono S, HR 5/5 column; mobile phase 0.05 M Bicine buffer, pH 8.4; linear salt gradient 0–0.35 M NaCl; flow rate 1.0 ml/min; ambient temperature; UV detection at 280 nm; sample size 7 mg dry weight. The linear salt gradient is illustrated as the dotted line. Identified enzyme activities are indicated in the gradient elution profile.

Fig. 2. Ion-exchange chromatographic separation of white-faced hornet. For conditions and details see Fig. 1.

on the use of Mono STM cation exchanger for studying bee venom and vespid venom. As the composition of the various vespid venoms appears to be similar, white-faced hornet vespid venom has been chosen as a model.

Experimental. Honey bee venom (*Apis mellifera*) obtained by electrical stimulation was purchased from Mr. C. Mraz, Middlebury, VT, USA. White-faced hornet (*Dolichovespula maculata*) was bought as lyophilized material from Dr. A. W. Benton, Pennsylvania State University, Spring Mills, PA, USA. The vespid venom was extracted from venom sacs.

Ion-exchange chromatography was carried out on an FPLC-system equipped with a cation exchanger Mono STM (HR 5/5, Pharmacia Fine Chemicals, Uppsala, Sweden).⁶ The venoms were dialyzed against starting buffer 50 mM Bicine [*N,N*-bis(2-hydroxyethyl)glycine], pH 8.4 prior to injection on the column. The venoms were eluted from the ion-exchange column by applying a linear salt gradient at constant pH.

The eluted material was monitored by UV absorbance at 280 nm.

Enzyme activities were measured on the collected fractions from the ion-exchange chromatography gradient elution profile as previously described.⁷

Results and discussion. Electrofocusing-electrophoresis titration curves⁸ were produced on bee venom and white-faced hornet vespid venom in order to establish a suitable pH for choosing proper conditions for cation-exchange chromatography on Mono STM.

Fig. 1 shows a gradient elution profile after ion-exchange chromatographic separation of bee venom components. The insect venom (7 mg) was adsorbed to the cation-exchanger in the starting buffer (50 mM Bicine) and elution was carried out at pH 8.4 with a linear salt gradient of NaCl at a rate of 8.75 mM/min ml. The various fractions from the elution profile were collected and analyzed with respect to the presence of enzyme activity. It is a well-known fact that the

predominant allergens in both bee venom and vespid venoms are enzymes.² As can be seen from Fig. 1 phospholipase A and B enzyme activities as well as acid phosphatase activity could not be separated and is detected in the fraction not adsorbed to the cation-exchanger at pH 8.4. After applying the salt gradient hyaluronidase was eluted as a symmetrical sharp peak (retention time 10 min). Obviously the applied experimental conditions give only a limited separation of bee venom components.

Fig. 2 shows a cation-exchange chromatographic separation of white-faced hornet vespid venom. The vespid venom (7 mg) was adsorbed to Mono STM in 50 mM Bicine buffer at pH 8.4 and eluted with a linear salt gradient (0–0.35 M NaCl) at a rate of 8.75 mM/min, ml. Various fractions were collected and analyzed with respect to the presence of enzyme activities. Acid phosphatase appears not to be adsorbed to Mono STM at pH 8.4. After applying the salt gradient several well-separated components giving absorbance at 280 nm were eluted. The first eluted peak (retention time approx. 5 min) exhibited hyaluronidase enzyme activity. After that a region with several small partly separated peaks follows (retention time 9 min) showing phospholipase B enzyme activity. The sharp peak (retention time 11 min) was identified as a protein but did not show enzyme activity. This peak might be the previously identified antigen 5, a basic protein in vespids which appears to be a major allergen for vespid venom sensitive individuals.⁴ After that a partly separated peak follows (retention time 13 min) giving strong phospholipase A enzyme activity. Phospholipase A enzyme activity was also detected in the late eluted shoulder (retention time 25 min). The two sharp peaks positioned in the region with retention time 18–21 min show phospholipase B enzyme activity.

The elution pattern (absorbance at 280 nm) of white-faced hornet vespid venom and bee venom show quite different peptide/protein profiles. This indicates that the applied ion-exchange system might be a procedure of differentiate between insect venoms and isolated individual venom components. The high resolution and short retention times of the used cation-exchanger (Mono STM) render this system a very powerful tool for rapid discrimination and quality control of Hymenoptera stinging insect venoms.

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Received February 21, 1983.