Journal of Chromatography, 209 (1981) 121–124
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,585

Note

Isotachophoretic characterization of stinging insect venoms

R. EINARSSON*
Analytical Research, Pharmacia AB, Box 181, S-751 04 Uppsala (Sweden)
and
U. MOBERG
Research Department, LKB-Produkter AB, Box 305, S-161 26 Bromma (Sweden)
(Received December 15th, 1980)

Venoms of common stinging insects, such as bees, yellow jackets, wasps and hornets, are complex mixtures of amines, peptides and proteins (enzymes) with both pharmacological and allergenic activities[1,2]. Honey bee venom has been extensively studied, which has resulted in the detailed biochemical characterization of most of the venom components[1,3,4].

Vespid venoms, on the other hand, have been subjected to few biochemical investigations, although King *et al.*[2] described biochemical and immunological studies of vespid venom proteins.

The use of modern biochemical separation techniques has made it possible to isolate individual venom components, and methods utilized for the purification and characterization of venoms from stinging insects of the order Hymenoptera are based on gel chromatography, ion-exchange chromatography and electrophoresis[2–4].

Isotachophoresis has been applied to the separation and quantitation of low-molecular-weight components[5] and proteins[6,7]. The high resolving power, in conjunction with the requirement for a small amount of sample, makes this technique very promising for the simultaneous separation and characterization of components in the venoms of stinging insects. In this paper an isotachophoretic procedure is described for studying bee and vespid venoms.

MATERIALS AND METHODS

Honey bee venom obtained by electrical stimulation was purchased from Mr. C. Mraz, Middlebury, VT, U.S.A. The venoms of wasp, white-faced hornet, yellow hornet and yellow jacket were purchased as lyophilized material from Dr. A. W. Benton, Pennsylvania State University, Spring Mills, PA, U.S.A. The vespid venoms were extracted from venom sacs. Melittin and bee venom phospholipase A_2 were obtained from Sigma, St Louis, MO, U.S.A., and histamine from E. Merck, Darmstadt, G.F.R.

The venoms were dissolved separately in distilled water to a concentration of about 1 %. Melittin, histamine and phospholipase A_2 were dissolved in distilled water to a concentration of approximately 0.1 %.

0021-9673/81/0000–0000/$02.50

The isotachophoretic analyses were performed with an LKB 2127 Tachophor (LKB, Bromma, Sweden), equipped with a 23-cm capillary for screening purposes. A 43-cm capillary was used for the identification of the peaks. The apparatus was equipped with a UV and a conductivity detector.

The leading electrolyte was 5 m*M* potassium hydroxide solution, adjusted to pH 6.0 with cacodylic acid (Pfaltz and Bauer, Flushing, NY, U.S.A.). To the leading electrolyte was added 0.2% (w/v) of hydroxypropylmethylcellulose (HPMC, Methocel 90 HG, 15,000 cps; Dow Chem., Midland, MI, U.S.A.) to minimize electroendosmosis. The terminating electrolyte was 5 m*M* creatine adjusted to pH 4.4 with hydrochloric acid. The spacer solution was 1 μl of 5% Ampholine. Carrier ampholytes of pH 6–8 were obtained from LKB.

The analyses were run at a constant current of 200 μA initially, which was decreased to 50 μA before detection (isotachophoretic equilibrium). The total time of analysis was 15–20 min, depending on the length of the capillary used. Samples of 2–5 μl were injected by means of a Hamilton syringe. The separated sample zones were detected by UV (transmission at 280 nm) and conductimetric detection. The transmission at 280 nm was recorded at a chart speed of 3 cm/min. The capillary was thermostated at 15°C.

RESULTS AND DISCUSSION

Fig. 1 shows the isotachophoretic separation of honey bee and vespid venoms in the presence of spacer solution (used in order to space the components according to

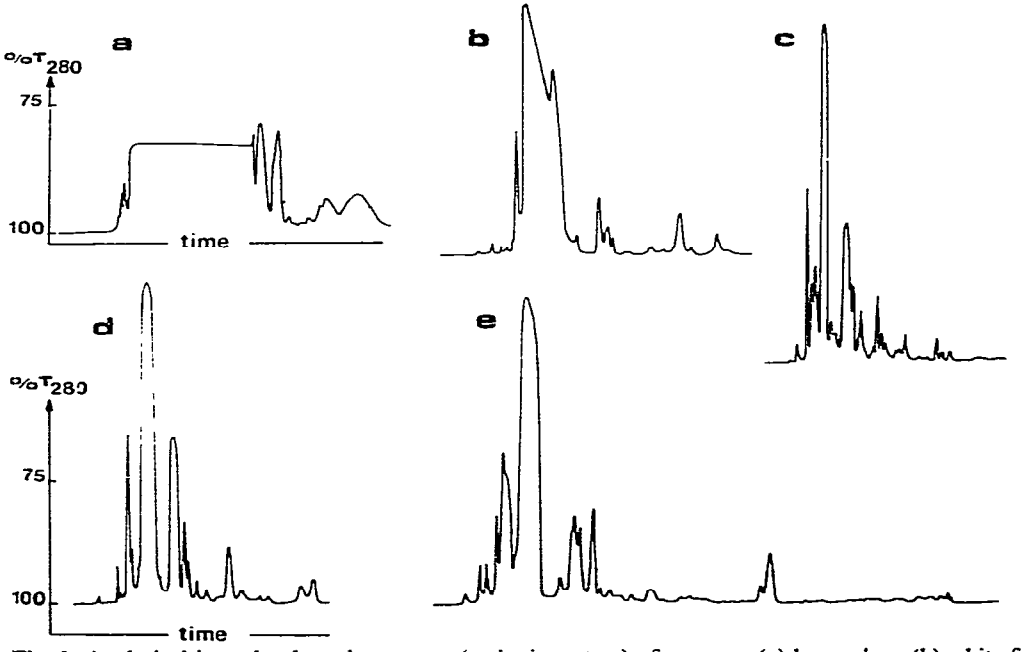

Fig. 1. Analytical isotachophoresis patterns (cationic system) of venoms: (a) honey bee; (b) white-faced hornet vespid; (c) wasp vespid; (d) yellow hornet vespid; (e) yellow jacket vespid. A 2-μl volume of each sample was injected with 1 μl of a 5% solution of spacer.

their mobilities), recorded with a UV detector (transmission at 280 nm). The UV profiles are characterized by a large number of absorbing zones (closely stacked peptide/protein patterns) for each venom species. On comparing the individual isotachophoretic patterns, it can be seen that honey bee venom (Fig. 1a) exhibits an isotachophoretic pattern with a major zone peak, which differs significantly from the vespid venoms. The long homogeneous zone in the honey bee venom profile indicates that one component is present in a large amount, as the zone width is directly proportional to the molar amount of an individual component. It is likely that this long zone is caused by melittin, as this peptide component constitutes about 50% of the dry weight of honey bee venom[1,2].

White-faced hornet, yellow hornet and yellow jacket venoms (Fig. 1b, d and e) exhibit similar isotachophoretic pattern, but wasp venom (Fig. 1c) shows a different profile, characterized by several well resolved zones. Inspection of the isotachophoretic profiles (fingerprints) of each venom species show, however, that it is possible to differentiate between the venoms investigated. Further, the results of several separate runs on each species of venom indicated very high reproducibility, which makes the isotachophoretic technique most suitable for routine identification purposes.

Fig. 2 illustrates the identification of the major component in honey bee venom. The isotachopherograms were recorded using both a UV and a conductimetric detector in the absence of spacer solution. The signal from both detectors demonstrates a high resolving power. Fig. 2a shows the separation of the complex peptide/protein mixture, and addition of melittin to the venom markedly increased the size of this homogeneous zone (Fig. 2b), which confirms that melittin is the major component.

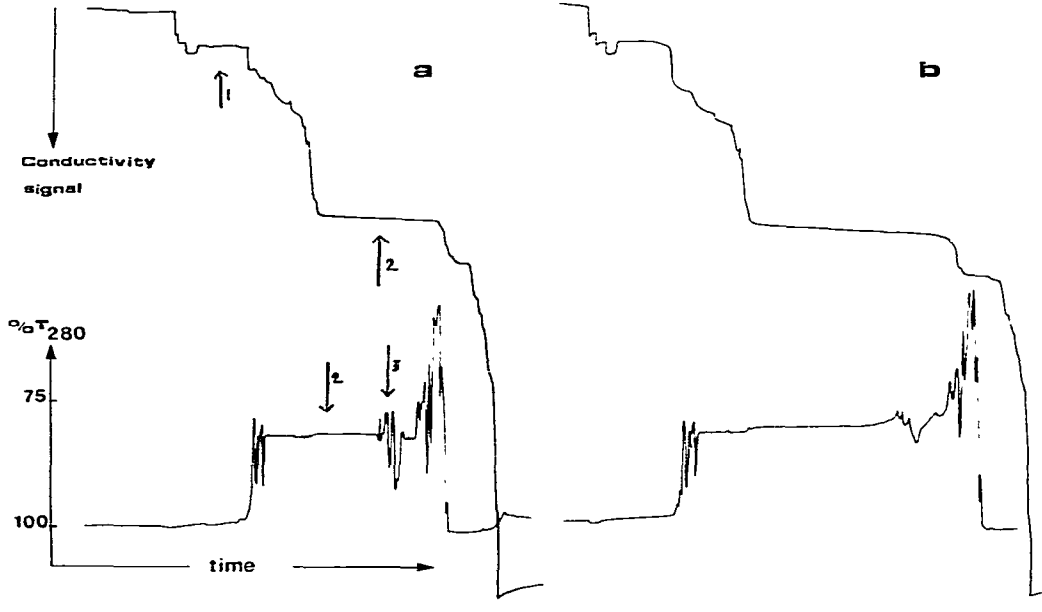

Fig. 2. Analytical isotachophoresis (cationic system) of (a) honey bee venom and (b) honey bee venom plus melittin. Arrows: 1 = histamine; 2 = melittin; 3 = phospholipase A_2. No spacer was used.

Further, the presence of histamine in honey bee venom has been verified. Because histamine does not absorb UV light, it must be detected with a conductivity detector, which is a general detector with high sensitivity (less than 50 pmol can be measured). Our studies indicate that histamine exhibits a high mobility in this electrolyte system and moves in front of melittin (Fig. 2a). Phospholipase A_2, on the other hand, showed a lower mobility than melittin (Fig. 2a). Further studies are in progress to quantify the individual components of hymenoptera venoms.

Analytical capillary isotachophoresis is easy to perform. Insect venom samples consisting of microgram concentrations in microlitre volumes can be analysed within 15–20 min. The results of these preliminary experiments suggest that this technique may provide a means for the rapid characterization of the venoms of stinging hymenoptera (fingerprints). Further, it might also give general information concerning the compositions of bee and vespid venoms.

REFERENCES

1 E. Habermann, *Science*, 177 (1972) 314.
2 T. P. King, A. K. Sobotka, A. Alagon, L. Kochoumian and L. Lichtenstein, *Biochemistry*, 17 (1978) 5165.
3 D. R. Hoffman, *J. Allergy Clin. Immunol.*, 59 (1977) 364.
4 T. P. King, A. K. Sobotka, L. Kochoumian and L. M. Lichtenstein, *Arch. Biochem. Biophys.*, 172 (1976) 661.
5 F. M. Everaerts, J. L. Beckers and T. P. E. M. Verheggen, *Isotachophoresis; Theory, Instrumentation and Applications*, Elsevier, Amsterdam, 1976.
6 P. Delmotte, *Sci. Tools*, 24 (1977) 33.
7 P. W. Lange, in A. Adam and C. Schots (Editors), *Biochemical and Biological Applications of Isotachophoresis*, Elsevier, Amsterdam, 1980, p. 187.