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Note

Application of high-speed aqueous gel permeation chromatography to insect venoms

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Gel filtration has been one of the most important techniques in biochemical research since its introduction[1] for separation and purification purposes and in the measurement of the molecular weight and distribution of water-soluble macromolecules. However, the matrices of the gels used are soft and weak, and consequently they do not withstand the high pressures required for high-speed analysis. A considerable amount of protein is needed, the separation procedures are often very time consuming and the resolution may be poor.

Many attempts have been described for the application of high-speed aqueous gel filtration of macromolecules[2]. However, many problems have been found, such as low column efficiencies and significant adsorption of the solute on the gel packing materials[2,3].

For the fractionation and analysis of insect venoms different gels have been used, such as Sepharose and various types of Sephadex[4–6]. In this paper we report on the use of a hydrophilic polyester packing (Shodex OHpak B-804/S) for aqueous gel permeation chromatography (GPC) for the separation and characterization of different insect venoms.

EXPERIMENTAL

Honeybee venom was purchased from Mr. C. Mraz, Middlebury, VT, U.S.A., and was obtained by the electric shock method. The venoms of wasp, white-faced hornet, yellow hornet and yellow jacket were purchased from Dr. A. W. Benton, Pennsylvania State University, Spring Mills, PA, U.S.A., as lyophilized material. The vespid venoms were extracted from the venom sacs.

High-speed aqueous GPC was performed on a chromatograph consisting of a Labotron pump, loop-type sample injector (Waters Assoc., Milford, MA, U.S.A.) and UV detector (Perkin-Elmer flow-through type). Shodex OHpak B-804/S gel was packed in a stainless-steel tube (250 × 8 mm) (Showa Denko, Tokyo, Japan). The eluent (0.02 *M* ammonium acetate buffer, pH 4.75) was generally delivered at 0.3 ml/min (2–3 kg/cm²). Insect venoms were dissolved in the eluent to a concentration of approximately 0.3–0.5%. The injection volume was varied between 0.05 and 0.2 ml. The effluent was monitored at 254 nm. Blue Dextran 2000 and alanine were

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used as the marker components for totally excluding (V_0) and totally permeating (V_t) molecules.

RESULTS AND DISCUSSION

The high resolving power obtainable with the Shodex OHpak column was demonstrated with five different insect venoms. Figs. 1–5 show typical chromato-

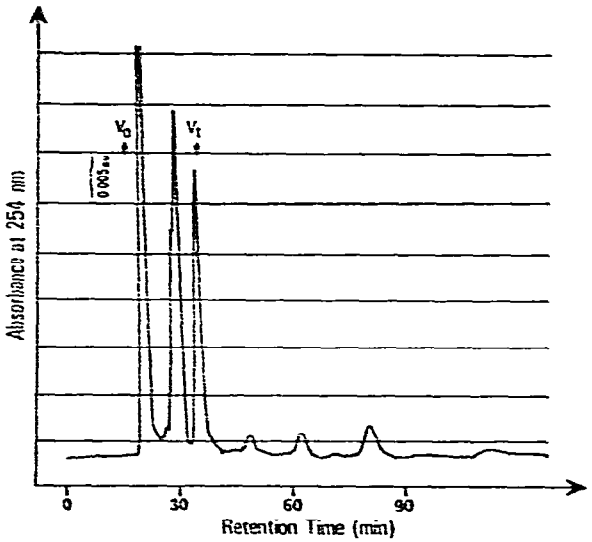

Fig. 1. High-speed aqueous gel permeation chromatogram of honey bee venom (986 μg). Operating conditions: packing, Shodex OHpak, B-804/S; column, 25 cm × 8 mm I.D.; mobile phase, 0.02 *M* ammonium acetate, pH 4.75; flow-rate, 20.0 ml/h; temperature, ambient; detection, absorbance at 254 nm.

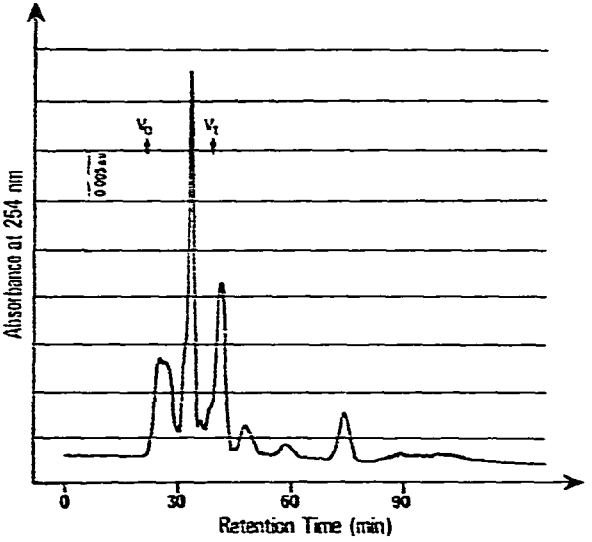

Fig. 2. High-speed aqueous gel permeation chromatogram of yellow jacket venom (648 μg). Operating conditions as in Fig. 1.

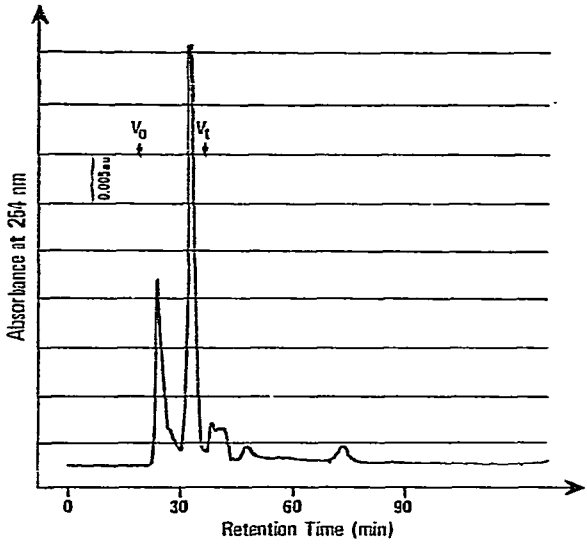

Fig. 3. High-speed aqueous gel permeation chromatogram of white faced hornet venom (500 μg). Operating conditions as in Fig. 1.

grams of the insect venoms eluted with 0.02 *M* ammonium acetate (pH 4.75). Samples of 0.1–1 mg of each insect venom species were applied to the column. On comparing the individual elution patterns it is evident that each species exhibits a characteristic elution profile and that the elution pattern is relatively independent of the amount applied and the flow-rate. Well resolved peaks eluted in the high-molecular-weight range appeared between V_0 and V_t, while peptides appeared in the

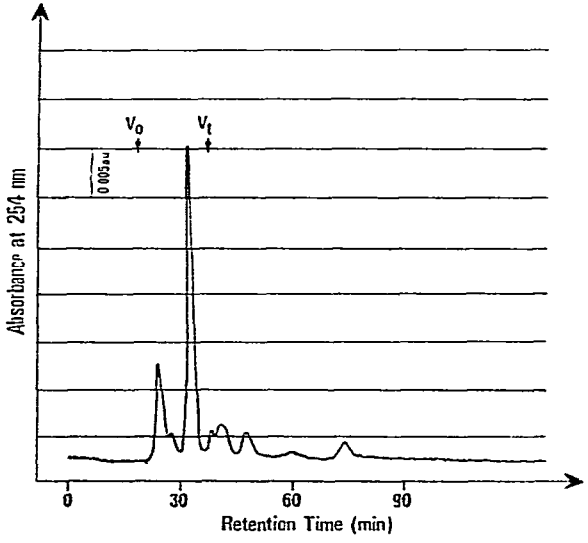

Fig. 4. High-speed aqueous gel permeation chromatogram of yellow hornet venom (390 μg). Operating conditions as in Fig. 1.

chromatogram after V_t, indicating significant adsorption on the matrix. This strong adsorption might be due to the fact that the protein/peptide components in the insect venoms studied are highly basic. Unlike the elution profiles obtained with conventional gel filtration[4–6], the protein/peptide peaks are well resolved in high-speed GPC. Results obtained to date suggest that it is possible to differentiate between the individual insect venoms tested provided that comparable concentrations are used. Further, the reproducibility of high-speed aqueous GPC is such that each species chromatographed on various occasions shows peaks occurring at similar retention times, rendering this technique suitable for identification purposes.

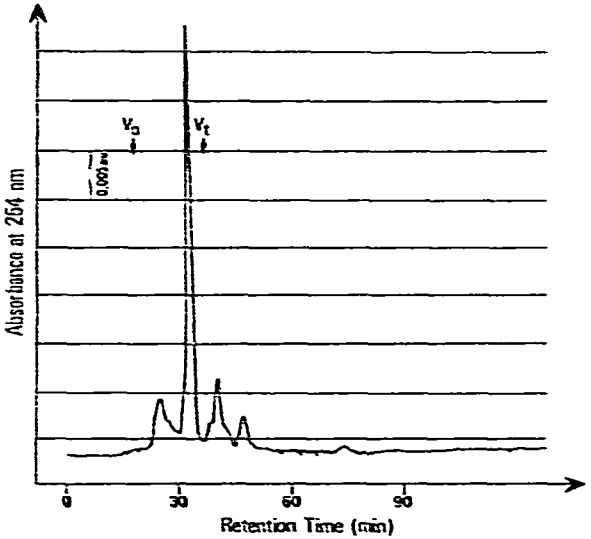

Fig. 5. High-speed aqueous gel permeation chromatogram of wasp venom (356 μg). Operating conditions as in Fig. 1.

These preliminary data show the potential use of Shodex OHpak columns in the hydrophilic aqueous GPC of insect venom proteins at low pressure. High-speed GPC with high resolution and sensitivity might greatly facilitate the increasing demands of the characterization of complex protein mixtures exemplified here by allergens.

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