

A HIGHLY PURIFIED ALLERGEN FROM EXCRETORY AND SECRETORY PRODUCTS OF *DIROFILARIA IMMITIS*

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Abstract—FUJITA K. and TSUKIDATE S. 1984. A highly purified allergen from excretory and secretory products of *Dirofilaria immitis*. *International Journal for Parasitology* 14: 547-550. Preceding data revealed that the allergen concentrated mainly in excretory and secretory (ES) products exhausted by adult *Dirofilaria immitis*. The present paper reported that a highly purified allergen was obtainable from ES products more easily and effectively. An allergen in ES products was purified by a combination of DEAE-Sephadex A-50 chromatography, Sephadex G-200 and Sephadex G-100 gel filtration. The purified preparation was proved to be one protein by sodium dodecyl sulfate (SDS) gel electrophoresis and to be exact the same allergen with the one obtained from the crude extract of adult *Dirofilaria* worms. The molecular weight of the purified allergen was estimated to be 15,000, and the allergen was inclined to aggregate in the buffered solution.

INDEX KEY WORDS: *Dirofilaria immitis*; allergen; antigenic analysis; ES products.

INTRODUCTION

NEMATODE antigens are particularly interesting, because the immunopathological reaction of the host to nematode infection is related to the potent allergenicity of the antigens (Haque, Quaissi, Joseph, Capron & Capron, 1981). Despite this potency, the allergen responsible for such activity has not been fully isolated and characterized.

Fujita (1975) separated allergen in crude aqueous extracts of *Dirofilaria immitis*, and Fujita & Tsukidate (1981) obtained a highly purified allergen. The allergen was proved to be a single protein, since it appeared as one band after sodium dodecyl sulfate polyacrylamide (SDS) gel electrophoresis and one precipitin arc by immunoelectrophoresis (Fujita, Ikeda & Tsukidate, 1979). The localization of the highly purified allergen was then studied with the fluorescent antibody technique. In this study, the specific localized site of the allergen was not observed clearly in the worm body itself, but excretory and secretory (ES) products derived from adult worms were determined to be the important source of the allergen (Fujita & Tsukidate, 1982). As the allergen was proved to be concentrated mainly in ES products, we tried to purify the allergen from ES products exhausted from adult *Dirofilaria* worms. In the present study, we could obtain a highly purified allergen from ES products more easily and effectively

than in the case of the purification of the allergen from extracts of worms (Fujita & Tsukidate, 1981).

MATERIALS AND METHODS

ES products. Adult *Dirofilaria immitis* worms were harvested from dogs in the Nagasaki area. One hundred adult male and female *Dirofilaria* worms were incubated in physiological saline at 27°C for 4 h, and ES products were obtained from incubation fluid. They were lyophilized and kept in a refrigerator until used.

Purification of allergen. Diethylaminoethyl (DEAE)-Sephadex A-50 (Pharmacia, Uppsala) was equilibrated in 0.015 M phosphate buffer, pH 7.2. Twenty millilitres of ES products were applied to a DEAE-Sephadex A-50 column (2.6 × 20 cm). Elution was carried out at room temperature with 0.015 M phosphate buffer, pH 7.2 containing stepwise increasing concentration of sodium chloride at (1) 0 M (starting buffer), (2) 0.125 M, (3) 0.25 M, (4) 0.5 M and (5) 1 M. Two millilitre fractions were collected at a flow rate of 0.2 ml min⁻¹. For the purification of allergen, the column of Sephadex G-200 (2.6 × 90 cm) (Pharmacia, Uppsala) was used. Sephadex G-200 was equilibrated in phosphate-buffered saline (PBS) at pH 7.2. Three millilitres of sample was applied, and gel filtration was carried out at room temperature with the same buffer at a flow rate of 0.2 ml min⁻¹. The effluent fractions were collected in 4.5 ml volumes. The protein peaks obtained were pooled separately, concentrated and dialysed with PBS. For the further purification of the allergen, gel filtration on Sephadex G-100 (1.6 × 100 cm) (Pharmacia, Uppsala) of the protein peak obtained was carried out in the same way.

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Assay of allergenic activity. Male ddY strain of mice were used for the detection of IgE antibody-inducing activity of fractions obtained by DEAE-Sephadex A-50 chromatography and by Sephadex G-200 or G-100 gel filtration of ES products. Five mice weighing 15–17 g were immunized with each fraction, and sera were examined with passive cutaneous anaphylaxis (PCA) and indirect haemagglutination (IHA) test as reported before (Fujita, 1975).

Analysis of antigenic composition. Antigenic analysis of each fraction was examined with polyacrylamide gel electrophoresis according to Davis (1964) and sodium dodecyl sulfate (SDS) gel electrophoresis according to Condeelis (1977). Electrophoresis was carried out in 7 cm long tubes per 90 min by applying a current of 5 mA per tube. Molecular weight of the purified allergen was estimated from the rate of mobility of the allergen to the maker of dye.

RESULTS

In order to separate allergenic component in ES products, 20 ml of ES products containing 150 mg of protein from adult *Dirofilaria* worms were applied to a DEAE-Sephadex A-50 column. The elution profile of ES products is shown in Fig. 1. Five peaks were obtained and they were designated as Fraction A, B, C, D and E in order of appearance. Five mice per group were immunized with $6 \mu\text{g} \times 2$ of each fraction, and the sera were examined with PCA and IHA test. The results showed that only Fraction B elicited positive PCA reaction without any IHA activity, whereas Fraction D had the ability to elicit only IHA activity, as shown in Fig. 1.

Antigenic analysis of Fraction B and D was tested by SDS gel electrophoresis, and antigenic composition of both fractions was compared with that of ES products. As shown in Fig. 2, Fraction B had one major band of lower molecular weight plus one or two bands of relatively higher molecular weight, whereas Fraction D had some higher molecular

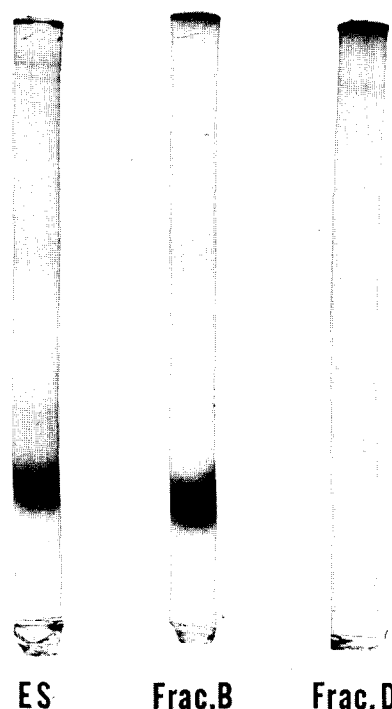


FIG. 2. SDS gel electrophoresis of ES products, and Fraction B and D obtained by the fractionation of ES products on DEAE-Sephadex A-50 chromatography.

bands. All of these bands shown in the SDS gel electrophoresis of Fraction B and D appeared to be the common bands with those from ES products.

The purification of allergen component from Fraction B was then carried out by gel filtration on

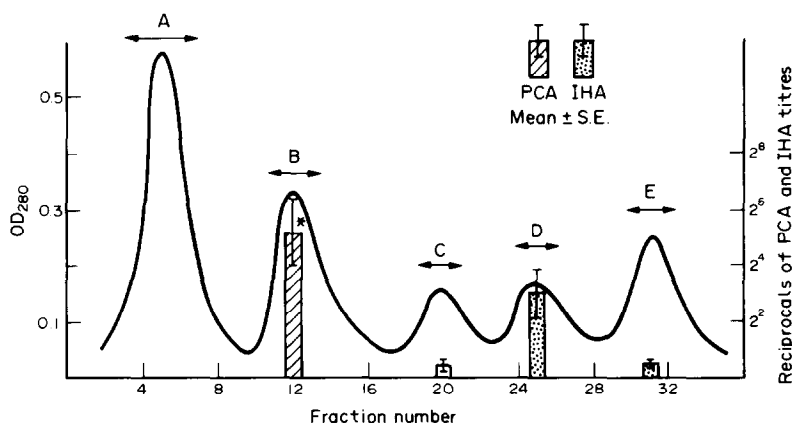


FIG. 1. Separation of the allergenic component in ES products exhausted by adult *Dirofilaria immitis* by chromatography on DEAE-Sephadex A-50. *Mean value and s.e. of the five mice immunized with $6 \mu\text{g} \times 2$ of Fraction B.

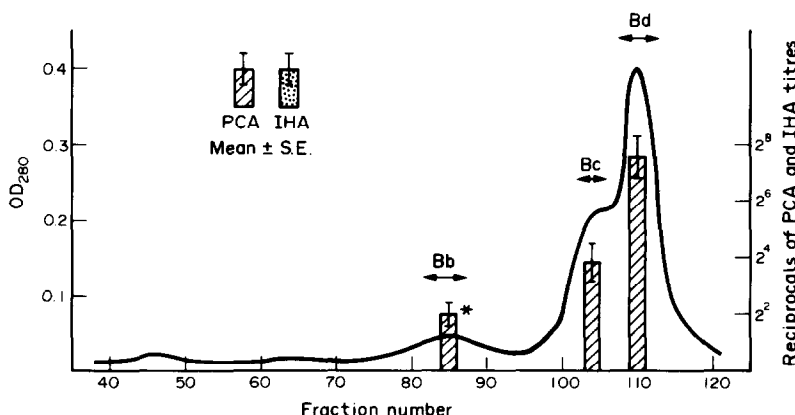


FIG. 3. Purification of the allergenic component in Fraction B by gel filtration of Sephadex G-200.

*Mean value and s.e. of the five mice immunized with $1.25 \mu\text{g} \times 2$ of Fraction Bb.

Sephadex G-200. Three millilitres of Fraction B containing 30 mg of protein was applied to the column, and three peaks were obtained. They were designated as Fraction Bb, Bc and Bd in order of appearance. Mice were immunized with $1.25 \mu\text{g} \times 2$ of each of these peaks. All the subfractions obtained from Fraction B elicited only PCA activity without any IHA activity in mice, but among them, Fraction Bd was the most active fraction for inducing PCA activity, as shown in Fig. 3.

Further purification of Fraction Bd was made by gel filtration on Sephadex G-100, and only one peak fraction was obtained. After SDS gel electrophoresis of this peak, only one band could be seen and the band was in good accord with the one of the highly purified allergen which was obtained from the crude extract of adult *Dirofilaria* worms (Fujita & Tsukidate, 1981), as shown in Fig. 4.

The molecular weight of this highly purified preparation was estimated by SDS gel electrophoresis. From the rate of mobility of the allergen to the maker of dye, the molecular weight of the allergen was determined to be 15,000. The highly purified allergen obtained from ES products showed one band in SDS gel electrophoresis as mentioned above, however, it appeared to have still many bands in ordinary disc electrophoresis, as shown in Fig. 4. The results suggested that the allergen was a single entity but aggregated or became attached to and formed complexes in buffered solution.

DISCUSSION

Parasite-derived ES products appear to be especially important, because ES products have been shown to stimulate protective immunity (Clegg & Smith, 1978), and they are known as better antigens for the specific diagnosis in helminth infection (De Savigny & Tizard, 1977; Weiss, Hussain & Ottesen, 1982). Although ES products are considered to be

particularly important, little is known about the nature of ES products. Irving & Howell (1982) characterized ES antigens derived from *Fasciola*

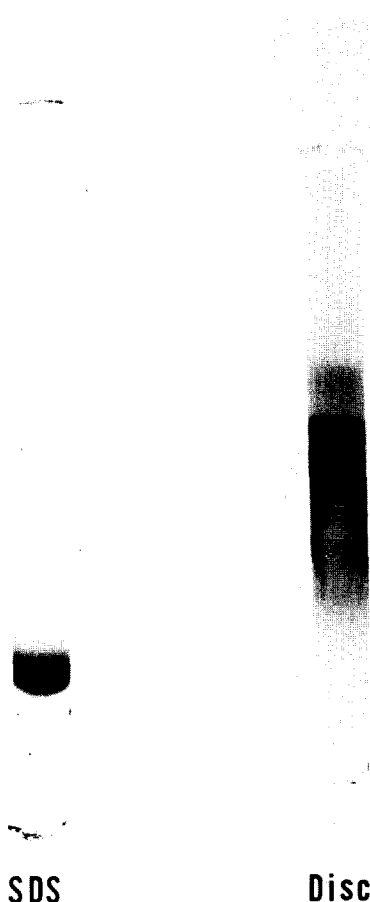


FIG. 4. SDS gel and ordinary disc electrophoresis of the purified allergen from ES products.

hepatica. Kaushal, Hussain, Nash & Ottesen (1982) studied ES products from adult *Brugia malayi* parasites and revealed 11 bands in the molecular weight range of 10,000–70,000 in ES products. They reported that, of the 11 ES bands, two appeared to be derived from the surface of the adult worms and microfilariae and another two bands were derived from host proteins. In our present study of the purification of the allergen from ES products, ES products from *D. immitis* appeared to have more bands by disc electrophoresis, but a relatively smaller number of bands were found when ES products were examined by SDS gel electrophoresis, suggesting that some of the antigens were inclined to aggregate in the buffered solution.

In the study of the localization of the allergen in *Dirofilaria* worms, we reported that the allergen concentrated in ES products and that it might be secreted and excreted from the digestive canal of the adult *Dirofilaria* worm (Fujita & Tsukidate, 1982). In the present study, we showed that the highly purified allergen was obtainable more easily and effectively from ES products exhausted from adult worms. The allergen which concentrated in ES products was purified in pure form in the present experiment and it was in good accord with the highly purified allergen from the extract of adult *Dirofilaria* worms. This observation may provide us with a handle to probe the mechanism of allergen–reagin interaction in the nematode infection.

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