Allergen from *Fasciola hepatica*; Separation of Allergen from IgG-inducing Antigens

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Abstract: Antigens of *Fasciola hepatica* adult worms were chromatographed using DEAE-Sephadex A-50 and Sephadex G-200. Two fractions (Frac. A_4 and Frac. B_4) which induced the highest titer of IgE antibody to the mice without any detectable indirect haemagglutinating antibody were obtained by these chromatographic methods. Antigen analysis revealed that these two fractions were antigenically same, and they were composed of one main allergenic component with small amount of other 7 to 8 component. These partially purified allergen had a molecular weight of 18,000 to 20,000, and they were inclined to aggregate in buffered solution. It seems that the allergen is located mainly in excretory and secretory products of *F. hepatica* adult worms.

INTRODUCTION

Human fascioliasis is known as an important zoonosis of foreign countries, especially of countries with many dairy farmers. However, only few cases of human fascioliasis (total 16 cases) have been reported in Japan. According to the latest report of Terui *et al.* (1973), reaginic reactions were observed in the patient during the course of the infection, and the intradermal test by using the crude antigen from adult *F. hepatica* was very useful for the diagnosis of human fascioliasis.

On the other hand, Hillyer and Capron (1976) reported that the crude antigen could detect quickly antibodies in cases of human fascioliasis but extensive cross-reactivity was seen between the crude extracted *F. hepatica* antigen utilized and the sera from humans with various parasitic infections. Most of this cross-reactivity was eliminated when a partially purified extract of *F. hepatica* was utilized as the antigen (Hillyer and Santiago, unpublished). This purification consisted of fractionation through Sephadex G-200 and pooling the crest and descending portion of the second peak and all of the

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third of four major peaks eluted from the column.

In order to get the purified allergen for diagnosis or for investigation purposes of fascioliasis, we conducted preliminary experiments and studied conditions for better production of IgE antibody in the mice immunized with the crude antigen extracted from *F. hepatica* (Tsukidate and Fujita, 1982). By using this effective system to induce IgE antibody in the mice, the purification of allergen from the crude extract was carried out in the present study.

MATERIALS AND METHODS

Preparation of crude extract (CE) of parasites: Parasites were harvested from cattle bile ducts in Kanazawa area and prepared as described in preceding paper (Tsukidate and Fujita, 1982).

Excretory and secretory (ES) antigen: ES antigen was obtained in the physiological saline at 37°C dipped with *F. hepatica* adult worms in 24 hrs.

Chromatography on DEAE-Sephadex A-50: Diethylaminoethyl (DEAE)-Sephadex A-50 (Pharmacia, Uppsala) was washed with distilled water and equilibrated in 0.015M phosphate buffer containing 0.01M 2-mercaptoethanol, pH 7.2. Fifteen milliliters of CE containing 600mg of protein was applied to 2.6×40cm column. Elution was carried out at room temperature with 24 to 30ml of 0.015M phosphate buffer, pH 7.2, containing stepwise increasing concentrations of sodium chloride at 0M (starting buffer), 0.125M, 0.25M, 0.5M and 1M. Five-milliliters of fractions were collected at a flow rate of 0.2 ml/min.

Gel filtration on Sephadex G-200: A 2.6×90cm column of Sephadex G-200 (Pharmacia, Uppsala) was equilibrated with PBS, pH 7.2. Five ml of the pooled fraction from DEAE-Sephadex A-50 was applied to the column. Gel filtration was carried out at room temperature with the same buffer at a flow rate of 0.2ml/min. The effluent fractions were collected in 5-ml volumes.

Animals: Male mice of outbred ICR at the age of 4 to 5 weeks were used throughout the experiment. Conventional Wistar rats of both sexes weighing 150g were also used. These animals were obtained from Hokuriku Lab. Inc., Kanazawa.

Immunization: ICR mice were immunized with 20γ of the pooled fraction eluted from DEAE-Sephadex A-50 or Sephadex G-200 column, and boostered with same antigen on 20th day. Wistar rats of male were also immunized with 1.5mg of the fraction eluted from DEAE-Sephadex A-50, and boostered on 5th day. These animals were injected intraperitoneally with the fraction mixed with aluminium hydroxide gel.

Estimation of protein: The protein content of each fraction was estimated by measuring UV absorption at 260mμ and 280mμ (Warburg and Christian, 1942).

Polyacrylamide gel electrophoresis: Disc electrophoresis was carried out with two kinds of polyacrylamide gels; one was ordinary polyacrylamide gel according to Davis

(1964) and the other was sodium dodecyl sulfate polyacrylamide gel (SDS-gel) electrophoresis according to Candeelis (1977). Both kinds of electrophoresis were carried out in 7cm long tubes for 90min by applying a current of 5mA per tube.

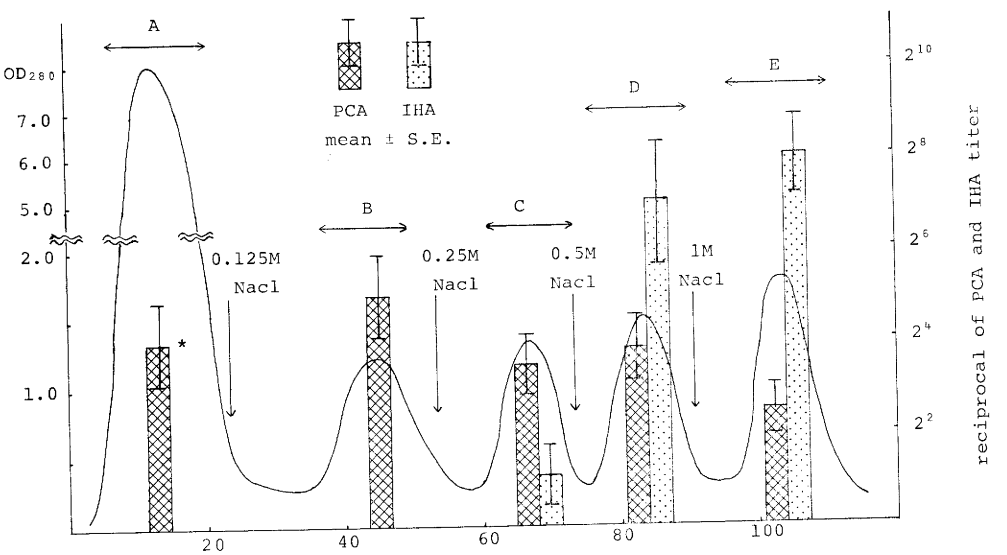
Passive cutaneous anaphylaxis (PCA) test and indirect haemagglutination (IHA) test: These tests were described in the preceding paper (Tsukidate and Fujita, 1982).

RESULTS

(1) Separation of allergen from IgG-inducing antigens.

(1-a) Antibody production of the mice immunized with the pooled fraction eluted from DEAE-Sephadex A-50 column.

In the preceding paper, it was determined that the most effective method to produce IgE antibody in the mice was to immunize with 20γ of CE and to booster on 20th day with same antigen concentration. ICR mice were immunized with the pooled fraction eluted from DEAE-Sephadex A-50 column. The CE from adult *F. hepatica* was applied to a DEAE-Sephadex A-50 column and fractionated as described in Materials and Methods. The elution profile is shown in Fig. 1. Effluent fractions of each of five separate peaks obtained by stepwise elution were pooled. Five mice per group were immunized



* mean value and S.E. in five mice immunized with 20γ of fraction A and boostered on 20th day.

Fig. 1. PCA and IHA antibody production of ICR mice immunized with fractions of CE by chromatography on DEAE-Sephadex A-50.

with each of fractions, and PCA as well as IHA tests were carried out with the sera obtained from the mice immunized. The results showed that fraction (Frac.) D and E elicited positive IHA test antibody activity with slight PCA antibody activity, and Frac. A and B induced only PCA antibody in this condition.

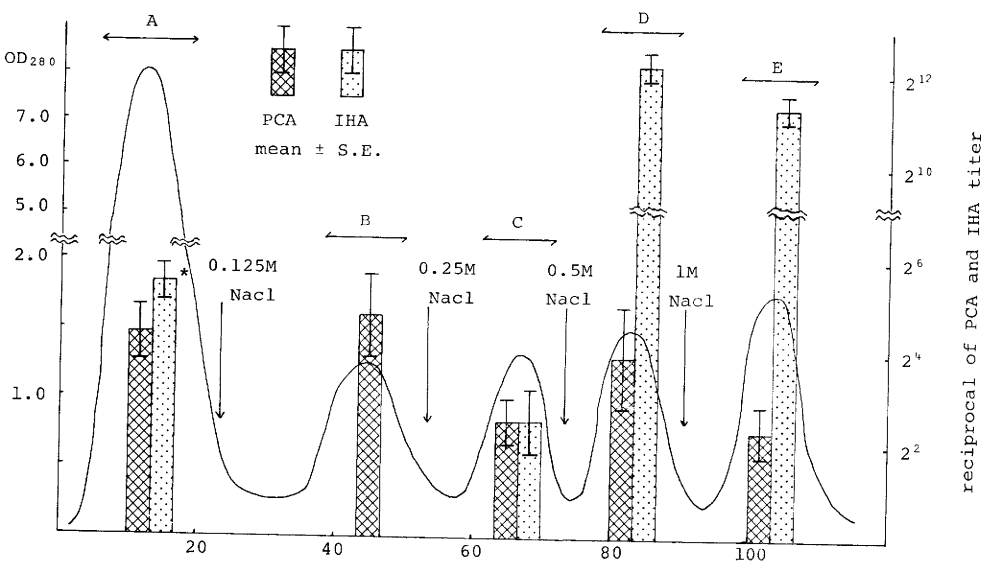
(1-b) Antibody production of the rats immunized with the pooled fraction eluted from DEAE-Sephadex A-50 column.

Separation of IgE-inducing antigens from IgG-inducing ones was successful in the mice by the immunization with the fraction eluted from DEAE-Sephadex A-50. In this time, the rats of Wistar strain were also immunized with the same fractions, and studied the localization of PCA antibody activity. As shown in Fig. 2, Frac. B induced only PCA activity but Frac. D and E induced very high titers of IHA activity with small activity of PCA. Different from the case of the mice, the rats accepted with Frac. A induced both PCA and IHA antibody activities.

(2) Partially purification of allergen.

(2-a) IgE antibody production of the mice immunized with the eluted fractions of Frac. A by Sephadex G-200 gel filtration.

The preceding data indicated that Frac. A and B contained mainly allergenic components to the mice. Further purification of Frac. A as well as B was carried out



* mean value and S.E. in four rats immunized with 1.5mg of fraction A and boostered on 5th day.

Fig. 2. PCA and IHA antibody production of Wistar rats immunized with fractions of CE by chromatography on DEAE-Sephadex A-50.

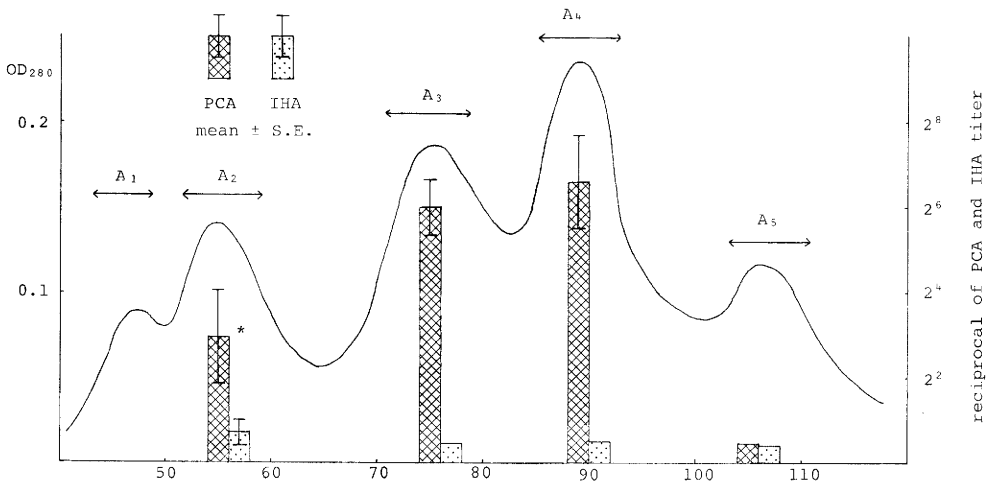
by the gel filtration on Sephadex G-200. As shown in Fig. 3, Frac. A was further separated into five peaks designated as A_1, A_2, A_3, A_4 and A_5. Frac. A_2, A_3 and A_4 had the ability to elicit PCA activity with very small titer of IHA antibody, however, Frac. A_4 induced the highest PCA activity in the mice.

2-b) IgE antibody inducing fractions of Frac. B by gel filtration on Sephadex G-200.

Frac. B was also subjected to gel filtration on Sephadex G-200 for further purification, and four peaks were obtained. The mice were immunized with each of fractions. PCA activity was elicited only by Frac. B_3 and B_4 and almost no IHA activity was induced by any of these fractions, as shown in Fig. 4. The mice immunized with Frac. B_4 induced the highest titer of IgE antibody.

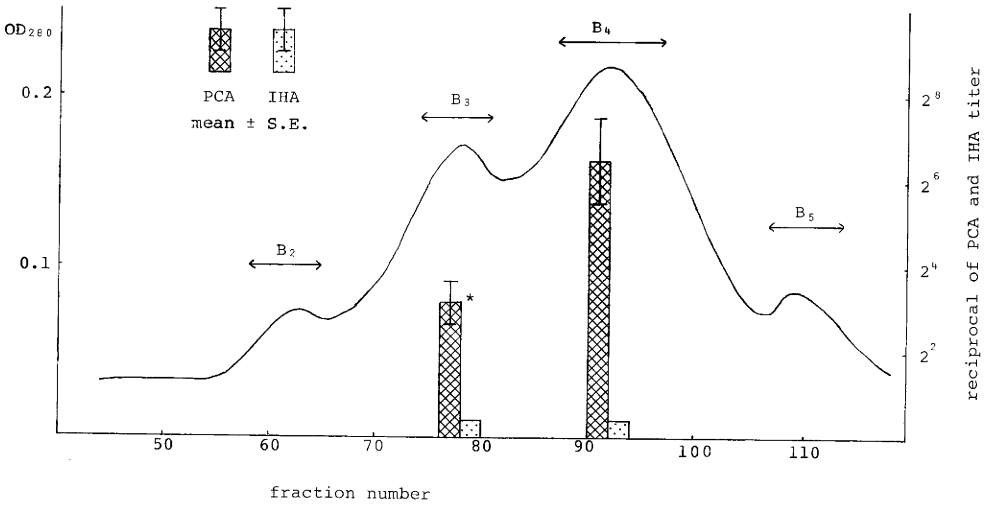
(3) Molecular weight estimation of the partially purified allergens.

Frac. A_4 and B_4 were considered to be the most active fractions inducing IgE antibody to the mice as judged from the preceding data. Molecular weight of these fractions was compared first. As shown in Fig. 3 and Fig. 4, Frac. A_4 was eluted on the same fraction number with that of Frac. B_4 by gel filtration on Sephadex G-200, and the molecular weight of both fractions was estimated on a calibrated Sephadex G-200 column. From the correlation between the elution volume and the molecular weight of reference proteins, the molecular weight of Frac. A_4 and Frac. B_4 was estimated to be the same, being approximately 20,000. The molecular weight of these fractions was also estimated by SDS-gel electrophoresis. From the rate of the mobility of the main



* mean value and S.E. in five mice immunized with 20γ of fraction A_2 and boostered on 20th day.

Fig. 3. PCA and IHA antibody production of ICR mice immunized with fractions of Frac. A through Sephadex G-200 gel filtration.



* mean value and S.E. in five mice immunized with 20γ of fraction B_3 and boostered on 20th day.

Fig. 4. PCA and IHA antibody production of ICR mice immunized with fractions of Frac. B through Sephadex G-200 gel filtration.

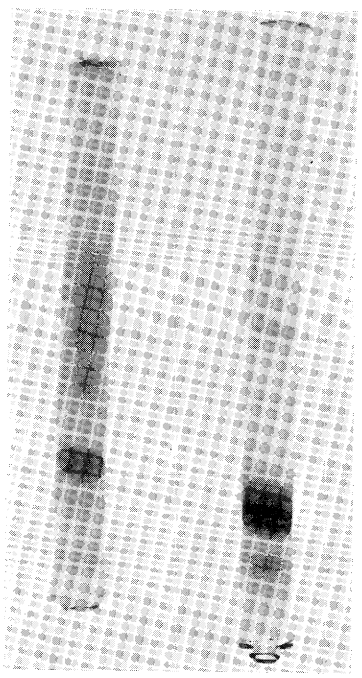


Fig. 5. Disc and SDS-gel eletcrophoretic patterns of the partially purified allergen (Frac. A_4).

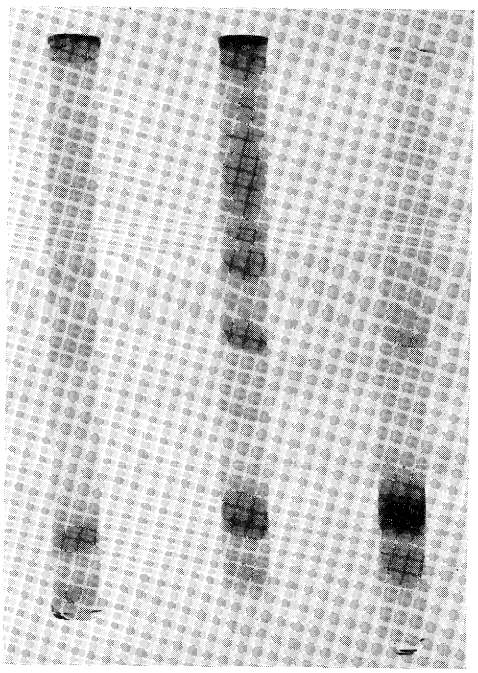


Fig. 6. SDS-gel electrophoretic patterns of CE of *F. hepatica*, ES antigen and the partially purified allergen (Frac. A_4).

component from Frac. A_4 and B_4 to the marker of dye, the molecular weight of the main component of these fractions was the same, approximately 18,000.

(4) *Antigenic analysis of the partially purified allergens.*

Disc as well as SDS-gel electrophoresis patterns of Frac. A_4 were examined in order to know the antigen composition. Many obscure protein bands could be seen in the ordinary disc electrophoresis, but in SDS-gel electrophoresis, one main band and other 7 to 8 faint bands could be observed, as shown in Fig. 5. Electrophoretic patterns of Frac. B_4 were also studied. Disc and SDS-gel electrophoretic patterns of Frac. B_4 were determined to be the same ones of Frac. A_4. The results above mentioned and the molecular weight study indicated that Frac. A_4 and B_4 were composed of the same allergenic substances.

Then, the antigen composition was compared among CE of *F. hepatica* adult worms, ES antigen and the partially purified allergen (Frac. A_4) by SDS-gel electrophoresis. As shown in Fig. 6, the main allergenic component appeared on the pattern of Frac. A_4 was shared by CE and ES antigen. Especially, ES antigen contained much amount of the allergenic component.

DISCUSSION

We had previously obtained the highly purified allergen from nematode worm, *Dirofilaria immitis*. The purified *Dirofilaria* allergen appeared as one band after SDS-gel electrophoresis and one precipitin arc by immunoelectrophoresis (Fujita and Tsukidate, 1981). The allergen had a molecular weight of 20,000 and a carbohydrate content of 2%, and it was resistant to the various kinds of chemical treatments as described in the previous paper (Fujita, Ikeda and Tsukidate, 1979). In the present report, the allergen from trematode worm, *F. hepatica* was studied. Although we could obtain only the partially purified allergen from *F. hepatica*, the main allergen component was very similar with that from *D. immitis*. Namely, the main allergen component of *F. hepatica* had the same electrophoretic mobility with that of *D. immitis*, and the molecular weight of the partially purified allergen was about 20,000, very similar with that of the purified allergen from *D. immitis*. The purified allergen was reported to be inclined to aggregate each other in the buffered solution. In the case of the allergen from *F. hepatica*, the same inclination was observed; many obscure protein bands were seen in the ordinary disc electrophoresis of the *Fasciola* allergen, but in SDS-gel electrophoresis of the allergen, only one main protein band and relatively small number of bands could be observed

Recently, the localization of the purified allergen in the *Dirofilaria* worm was clarified by us. The purified allergen was localized in the excretory and secretory products of adult *Dirofilaria* worm (Fujita and Tsukidate, unpublished data). Therefore, the antigen composition was compared between the partially purified allergen and ES antigen from *F. hepatica*. ES antigen was proved to have much amount of the main

allergen component, and the allergen was thought to be localized in ES products also in the case of *F. hepatica*.

Bout *et al.* (1977) demonstrated two kinds of allergens in adult *F. hepatica* extracts by means of radioimmunoelectrophoresis. One of them was identical to a species-specific antigen for genus *Fasciola* to interact with IgE antibodies of patients with fascioliasis. Another allergen component resembled the one described by Korach and Benex (1971). It was also shown to be a lipoprotein by means of Sudan black staining. We still could not obtain the purified allergen from *F. hepatica*, but the correlation of our allergen to these two allergens should be made clear in future.

An increasing body of evidence supports the concept that the mice immunized with antigens of *F. hepatica* aquire the immunity to challenge infection with a different parasitic trematode, *Schistosoma mansoni* (Christensen *et al.*, 1978; Hillyer and Sagramoso, 1979; Rajasekariah and Howell, 1978). Hillyer and Sagramoso (1979) demonstrated that *F. hepatica* glycoprotein antigens induced in mice the significant protection to the infection with *S. mansoni*. On the other hand, it has been suggested that IgE antibodies might play a role in immunity to parasites (Ogilvie *et al.*, 1966), and recent studies have indicated that IgE antibodies specific for *S. mansoni* play a significant role in adherence of normal macrophages to *S. mansoni* shistosomules and their killing the parasites (Capron *et al.*, 1975).

The separation of allergen from *F. hepatica* could favor future progress in immunoprophylaxis of these parasitic diseses and also in immunodiagnosis.

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肝蛭のアレルゲン
アレルゲンの部分精製とその抗原分析

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肝蛭成虫内に含まれているアレルゲンを，DEAE-Sephadex A-50 と Sephadex G-200のクロマトグラフィーによって，IgG 誘導抗原より分離・精製した．クロマトグラフィーで分けられた分画をそれぞれマウスに免疫することによって，高タイターの IgE 抗体を誘導する 2つの分画 (Frac. A_4 および Frac. B_4) を得た．SDS-gel 電気泳動法による抗原分析の結果，両分画の抗原組成は同一で，これらの分画は分子量18,000から20,000の1種類のアレルゲン分画と，他に極く微量の7ないし8種類の蛋白分画から成っていることが判明した．Disc電気泳動法で観察すると，このアレルゲンは，溶液中では極めて重合ないし凝集しやすい性質を有していることが想像された．このアレルゲン分画は，主として肝蛭成虫が排泄・分泌する，いわゆるＥＳ抗原中に存在していることが考えられる．

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