

## ALLERGENIC ACTIVITY OF FISH EXTRACT

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### ABSTRACT

A component of Fish extract has been isolated by a combination of 65% ammonium sulphat salt precipitation and gel filtration. The purified allergen appeared as two major peaks. Peak 11 exhibited the highest IgE binding inhibition and showed a specific allergenic activity about two fold higher than that of the peak 1. Peak 1 and peak 11 cross reacted in IgE binding inhibition. Completely inhibiting the binding of specific IgE to each other. The allergency of purified allergen was not lost during purification process.

### INTRODUCTION

For the estimation of airborne exposure to allergens or the amount of allergenic potency of allergen extract , the measurement of specific allergens in weight units is a feasible alternative . Almost all quantification method of allergens are based on the use of specific antisera. The simplest methods are radial immunodiffusion and rocket immunoelectrophoresis which require monospecific antisera . In the radial immunodiffusion , the size of the precipitation circle is proportional to the amount of a single allergen <sup>(1)</sup> . In the rocket immunoelectrophoresis , the height of the precipitate depends on the amount of single allergen<sup>(2)</sup> . Radioallergosorbent test (RAST) is a test for the measurement of allergen – specific IgE<sup>(3)</sup> . It can be used in the allergen quantification when the binding of patients IgE to the solid – phase allergen is inhibited by the same allergen (RAST – inhibition)<sup>(4)</sup> . The amount of the allergen is calculated from the ratio of 50% inhibition points of the sample and the standard curves. The ELISA-inhibition is anon radio active method based on the same principle . Depending on the assay design of inhibition methods, it is possible to measure all allergens in a crude extract or a single allergen<sup>(5)</sup> . The aim of this study was

therefore to determined the allergic activity of fish extract and the purified allergen extract using the ELISA inhibition .

## MATERIALS AND METHODS

### **Sera:**

#### **Normal sera:**

Sera of 20 healthy subjects from Basrah University, who were free from any clinical symptoms and who were skin-prick test (SPT) and ELISA negative to fish.

#### **Allergic sera:**

Twenty sera from subject sensitive to fish extract (as proved by SPT, ELISA and clinical history)<sup>(5)</sup> were also used in this study. All subjects had a history of asthma and rhinitis.

#### **Antigens:**

Aqueous extract of fish *Tenualosa ilisha* was prepared from the meat of raw fish which was ground in PH 7.2 phosphate buffer saline PBS at 1: 20 ( W / V) ratio in awarring blender , extracted overnight under refrigeration and centrifuged at 10000 rpm for 1hour at 4 C<sup>(6)</sup> . The supernatant was desalted by gel filtration on sephadex G-25 and stored at 4C<sup>(8)</sup> .The protein content was estimated by the method of Whitaker and Granum<sup>(7)</sup>.

#### **Antibodies:**

Rabbit antihuman Ig and horseradish peroxidase had been conjugated as previously reported<sup>(8,5)</sup>.

#### **ELISA Inhibition:**

For competition ELISA microtiter 96 well plates were coated with allergen extract at 100 u.g/ml for 1 hour at 37 °C<sup>(6)</sup> .The plates were then blacked for 1 hour with (PBS-BSA)/ phosphate buffered saline Bovine serum albumin 1 %. Afterwards 25 µl of the serum (diluted 1:3 in PBS) and 25 µl of serial dilutions of the allergens extract were added to the wells and incubated for 2 hours at room temperature. After washing wells were incubated with peroxides labeled atnihuman IgE for one hours at room temperature. The plates were washed again and developed by incubating with 50 µl/well of solutions of orthophenylen diamine (OPD) for 30 minutes. The reaction was stopped by adding 50 µl of

2 M HCl/td and the absorbance was measured at 492 nm. Assays were performed in duplicate.

## RESULTS

### Allergenic activity of the purified allergen

On fractionating fish, two major peaks were observed (Fig. 1), the allergenic activity of these two peak (peak I and peak II) and the crude protein extract was evaluated by means of competition ELISA as have been shown in table 1; either peak I and peak 2 could inhibit to a high extend; the binding of specific IgE to the whole protein extract (peak 1 72 % and peak 2 73 %); suggesting that these protein bear major allergenic determinant of the source material extract; The upper value in each entry mean the amount of protein in micrograms per well needed to reach 50 % inhibition. The lower values represent the maximum inhibition reached (%) furthermore. The IgE binding capacity of peak 2 protein was found to be higher than that of peak 1 protein; since lower concentration of peak 2 protein was needed to inhibit up to 50 % the binding of specific IgE to the allergosorbant phases.

**Table 1. cross-inhibition of specific IgE, binding among Fish extract (FE). Peak I and Peak II, using ELISA inhibition**

Allergosorbant	Inhibitor		
	Peak II	Peak I	Peak II
Fish extract	1.0/73	1.9/72	1.0/73
Peak I	0.6/90	1.6/96	0.6/90
Peak II	0.9/93	1.8 /89	0.9/93

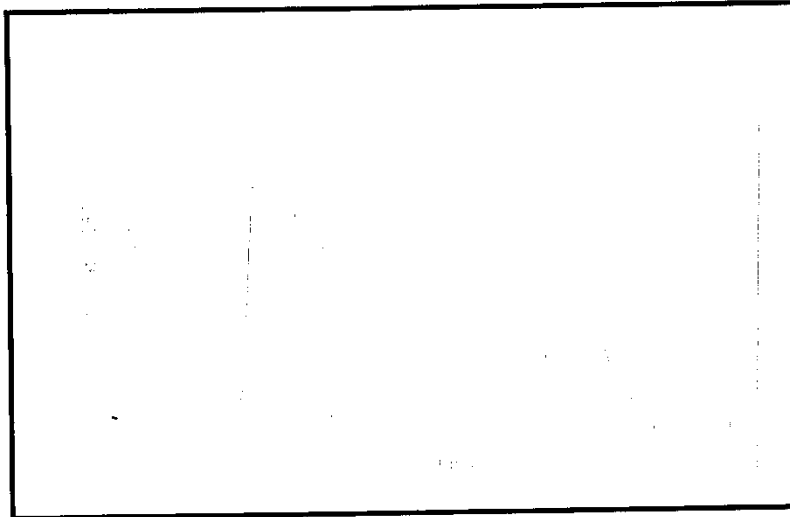


Fig. 1: Elution profile of fish protein.

#### DISCUSSION

As far as the allergenic activity of protein in peak 1 and peak 2 is concerned data presented in this work clearly evidence that protein of these peaks are the most clinically relevant allergens from the tested source materials Besides, allergens of peak 1 and peak 2 independently accounted for a high percentage (about 75 %) of the total allergenic activity. Demonstrating that they are the allergens from their source material and consequently other allergens might be present in the extract with a little allergenic importance in comparison with that of peak 1 and peak 2.

On the other hand peak 1 and peak 2 cross-reacted in IgE-binding inhibition completely inhibiting the binding of specific IgE to each other. It seems clear that both protein bear the same allergenic epitopes and this fact supports the idea of extremely high homology between them. This finding are in line with those previously reported<sup>(10)</sup> that cross reactions

may extend to the presence of carbohydrate structures shared by several components of the same protein extract. Nevertheless, a slight difference between these peaks was observed for the amount of protein needed to inhibit up to 50 % the binding of specific IgE to each other or to whole extract, about two fold peak 1 to peak 2 amount were necessary to reach similar inhibition levels.

The allergenicity of isolated peaks was not lost during the purification process since it maintained the ability of inhibiting the binding of specific IgE from sensitive subjects. This finding was in line with that previously reported<sup>(11)</sup>.

In conclusion our data support the hypothesis that the allergenicity of purified allergen was not lost during the purification process.

#### الفعالية المستأرجية لخلصة السمك

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#### الخلاصة

عزلت مكونات خلاصة السمك بواسطة الترسيب بكبريتات الأمونيوم (65%) والترشيح الهلامي . ظهر المستأرج المنقى متمثلاً بقيمتين كبيرتين هما القمة 1 ، 2 . القمة 2 أظهرت قدرة عالية على تثبيط ارتباط IgE وكذلك فعالية مستأرجية أعلى بقدر الضعف مما هو موجود في القمة 1 . يوجد تفاعل تصالبي بين القمتين في تثبيط ارتباط IgE بكل واحدة منها وأن الفعالية المستأرجية للمستأرج النقي لا تفقد أثناء عملية التنقية .

#### REFERENCES

1. Ingilg . A (1,83- single radial immunodiffusion . In: Axelsen . N. H.(ed) – Handbook of immunoprecipitation. In: gel techniques. Scand. J. Immunol. 17:41 – 56 .
2. Axelsen ; N.H and Bocle . E ( 1,83). Electro immunoassay (Rocket immunoelectrophoresis . In Axelsen . N. H. edy. Hand book of immunoprecipitation. In Gel Techniques. Scand . J. Immunol. 17 : 1103 – 106.
3. Ceska, M. and lundysvist, U. (1,72). A new simple radioimmunoassay method for the determination of IgE. Immunochem . 9: 1021 – 1030.

4. Yman, J.; Ponterins, G. and Bran, H. (1975). RAST based allergen assay methods. Dev. Biol. Stand. 29: 151 – 165.
5. Abdulla, F.A. (2001). Study of some local allergens, partial purification, characterization and preparation of allergy vaccines. P.h.D. thesis, Sci – Coll. Basrah University. P. 34-47.
6. Yanginger, J. W.; Sweeney, K. G.; Stumer, W. O. and Helm, R. M. (1988). Fatal food induced anaphylaxis. JAM Med. Assoc. 260: 1450.
7. Whitakar, R. J. and Granum, E. P. (1980). An absolute method for protein determination based on difference in absorbance at 235 and 280 nm. Analytical biochemistry. 109: 156-159.
8. Leslie, H. and Frank, C. H. (1976). Isolation and structure of immunoglobulins in: Practical immunology 3<sup>rd</sup> edition; lack well scientific Publication, Oxford, London.
9. Jaime, M.; Lucia, J.; Teadora, O. and Jose, C. (1991). Monoclonal antibodies against human IgE: identification of an epitope sharing properties with the high-affinity receptor binding site. Molec. Immunol. 28:839-841.
10. Gonzales, R.; Palo, F.; Zapater, L. and Carreira, J. (1992). Purification and characterization of major inhalant allergen from soybean hulls. Clin. Exp. Allergy. 22: 748-755.
11. Ayuso, R.; Palo, F. and Carreira, J. (1988). Purification of par J1, the major allergen of *Pariefariajundica* Pollen. Mol. Immunol. 25: 49-56.