

Immunoblot Analysis of IgE-Binding Antigens in Spices

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Abstract. The IgE response to coriander and other spices was studied by immunoblotting, after separation of the spice extracts by isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A major IgE-binding component from coriander had an isoelectric point of pH 5. After incubation of SDS-PAGE-separated spice extracts with serum from a patient with an occupational allergy to spices, a closely related pattern of IgE binding to coriander, dill and anise extract was observed. These results suggest that the botanically related spices, coriander, anise and dill, contain common IgE-binding structures.

Since its introduction in 1967 [1], the radioallergosorbent test (RAST) has become an important tool in allergy diagnosis. In the RAST, free circulating allergen-specific IgE antibodies are determined with the use of solid-phase coupled allergen extracts [1]. Such allergen extracts are complex mixtures of proteins and carbohydrates; only a few components will elicit an IgE response in allergic patients [2, 3]. With the use of isoelectric focusing (IEF) and polyacrylamide gel electrophoresis (PAGE), complex mixtures of proteins can be fractionated according to isoelectric point [4] or molecular weight [5]. With the technique of immunoblotting [6], the heterogeneity of antibody-binding structures in complex mixtures like allergen extracts can be studied [7-9]. We recently described the presence of IgE antibodies directed against various spices in a patient with occupational asthma induced by inhalation of dust from spices [10] and in patients with symptoms of food allergy [11]. Peltre et al. [12] and Pauli et al. [13] studied IgE-binding structures in celery root by immunoblot analysis.

In the present study we analyzed the IgE-binding structures in some other spices using immunoblotting techniques.

Most experiments were performed with serum from patient M, who has been described previously [10]. Patient M developed occupational asthma while working with spices in the food industry. In the serum of patient M, very high levels of spice-specific IgE

were present [10]. Other sera with IgE against coriander and other spices were from patients with symptoms of food allergy like urticaria, angioedema and/or eczema [11].

Table I shows the results of the spice RASTs for those sera that were used in the immunoblot experiments. Total IgE in sera was determined by IgE radioimmunoassay (Pharmacia, Uppsala, Sweden) according to the manufacturers' instructions. IgE against spices was measured as described previously [10, 11].

Powdered spices were obtained from a local grocery. The spices used were coriander (*Coriandrum sativum*), ginger (*Zingiber officinalis*) and curry. Curry is a mixture of various spices: its most important compo-

Table I. Measurement of IgE against spices in 4 sera

Patient No.	Curry	Coriander	Ginger	Total IgE IU/ml
1 (M)	44	45	27	4,000
2	17	18		1,200
3	14	13		2,000
5	21	22		1,100

The results are expressed as percent binding of ¹²⁵I-anti-IgE.

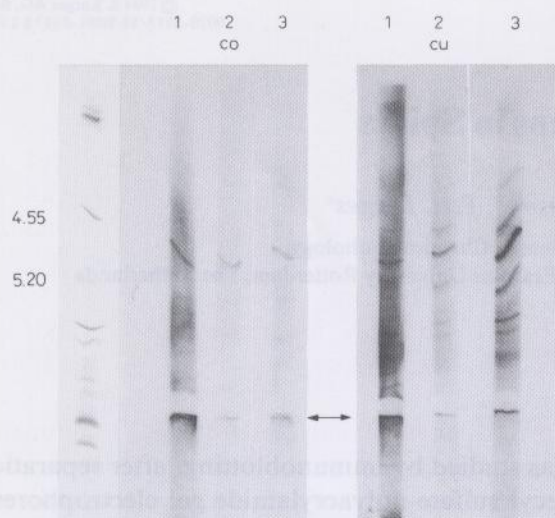


Fig. 1. IgE binding to extracts of coriander (co) and curry (cu), separated by IEF and transferred to nitrocellulose. See table I for patient data. The left lane shows IEF-separated pI markers, stained with Coomassie blue. The arrow indicates the point of application of the spice extracts.

nents are coriander, chilli pepper and black pepper [14]. Whole seed of dill (*Anethum graveolens*) and anise (*Pimpinella anisum*) were ground before use. For analysis by electrophoresis 100–200 mg powdered spices were extracted with 1 ml distilled water for 1 h at room temperature under agitation. After centrifugation the supernatant was stored in small aliquots at -20°C . The protein content of these extracts, estimated according to Schacterle and Pollack [15], ranged from 2.5 to 4 mg/ml. Per electrophoretic run 0.025–0.030 ml spice extract was used.

Agarose gel IEF was performed according to Bengtsson et al. [9]. Sodium dodecyl sulfate-PAGE (SDS-PAGE) was performed according to Laemmli [5]. Before SDS-PAGE, spice extracts were mixed with an equal volume of sample buffer and heated for 5 min at 100°C (sample buffer: 0.125 M Tris HCl, 4% SDS, 20 mM dithiotreitol, 20% glycerol and 0.005% Bromphenol blue, pH 6.8). Low molecular weight markers and pI isoelectric point markers were obtained from Pharmacia. Proteins in polyacrylamide gels were stained with Coomassie brilliant blue (CBB) G 250; proteins in agarose gels were stained with CBB R 250.

The separated spice extracts were transferred from the separation gel to nitrocellulose membranes

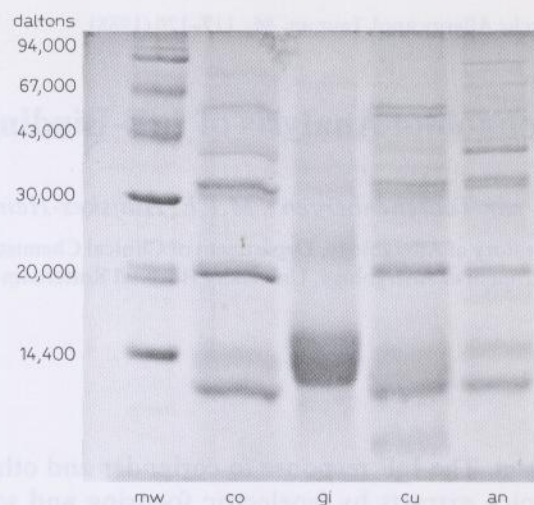


Fig. 2. Coomassie blue staining of spice extracts, separated by SDS-PAGE. co = Coriander; gi = ginger; cu = curry; an = anise; mw = molecular weight.

(Transblot nitrocellulose, $0.45\ \mu\text{m}$, Biorad, Utrecht, The Netherlands). After IEF, transfer to nitrocellulose was accomplished by the immunoprint technique [7, 9]. After SDS-PAGE, transfer to nitrocellulose was done by electroblotting [6, 16]. After completion of the transfers from agarose or polyacrylamide gel, unoccupied binding sites on the nitrocellulose membranes were blocked by incubation with Phadebas RAST buffer (Pharmacia) with 0.5% Tween 20 for 1 h at 37°C . The nitrocellulose membrane with transferred proteins was cut into strips of 0.5 cm width. These strips were placed in incubation trays and incubated overnight at room temperature with 0.35 ml patient serum (0.075 ml in experiments with serum from patient M), diluted in RAST buffer, in a final volume of 1.5 ml. After 3 washes with saline, 0.5% Tween 20, radioiodinated anti-IgE antibodies (Pharmacia) were added.

After an incubation of 4 hours and three washes with saline, 0.5% Tween 20, the strips were air-dried and exposed to Kodak direct exposure film DEF-392 for 1–2 weeks for detection of ^{125}I -labeled bands.

When the 8 sera containing IgE antibodies against coriander were incubated with IEF-separated coriander extract, the same pattern of IgE binding was seen with all 8 sera tested. Figure 1 shows the results for 3

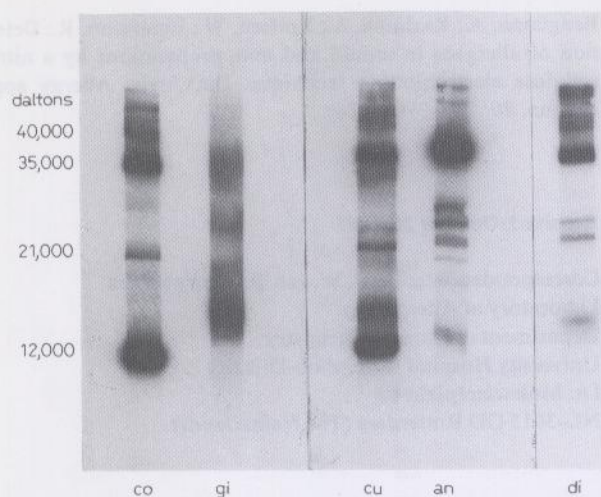


Fig. 3. IgE binding from serum of patient M to spice extracts, separated by SDS-PAGE and transferred to nitrocellulose. co = Coriander; gi = ginger; cu = curry; an = anise; di = dill. The molecular weight (daltons) of four IgE-binding components is indicated on the left side.

sera; IgE antibodies against coriander were mainly directed against a component with an isoelectric point at pH 5. An IgE-binding structure with an isoelectric point at pH 5 was also present in curry (fig. 1). Coriander is a major constituent of curry, which is a mixture of various spices [14]; indeed, more IgE-binding bands were seen in curry extract than in coriander extract. After incubation of IEF-separated coriander and curry extract with serum from a patient without symptoms of food allergy, no IgE binding was observed (data not shown).

The protein spectrum of spice extracts, after SDS-PAGE and CBB staining, is shown in figure 2. Immunoblotting after SDS-PAGE showed IgE binding to many bands with molecular weights in the range of 12,000–90,000 daltons. An IgE-binding band with a molecular weight of about 35,000 daltons could be observed in curry, dill, coriander and anise (fig. 3).

A band with a molecular weight of 12,000 daltons showed strong IgE binding with curry and coriander extract; this band was also seen after immunoblotting of dill and anise extract. Other IgE-binding bands, common to most spices, had molecular weights of 21,000 and 40,000 daltons. The SDS-PAGE separation of spice extracts was run under dissociating conditions. It is possible that figure 3 depicts IgE binding

to subunits of the native allergen molecule(s), after its dissociation by SDS-dithiothreitol. The protein spectrum (fig. 2) and the IgE-binding spectrum (fig. 3) of ginger extract was clearly different from the pattern seen with the other spices tested. Coriander, dill and anise belong to the plant family of the *Umbelliferae*, whereas ginger belongs to another family, the *Zingiberaceae*.

Serum from a patient without symptoms of food allergy showed no IgE binding to PAGE-separated extracts of coriander, ginger, curry, anise or dill extract (data not shown).

Figure 3 suggests that the botanical relationship between coriander, anise, dill and celery expresses itself in a related pattern of IgE binding. This indicates that, in all these spices, related structures exist that preferentially will elicit an IgE response. Knowledge of IgE-binding structures, common to various spices and other allergens [11], may further improve the diagnosis and management of food allergic reactions.

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