

Purification of Allergens in *Cladosporium herbarum*

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Extracts of *Cladosporium herbarum* were characterized by means of crossed immunoelectrophoresis (CIE) and the allergens were identified by means of crossed radioimmunoelectrophoresis (CRIE) (1). Two of the allergens (Ag-32 and Ag-54) were isolated by means of ultrafiltration, gel filtration and isoelectric focusing (3). The present communication deals with selected aspects of this work.

We at present characterize allergens in crude extracts as "major", "intermediate" or "minor" allergens as defined by their IgE binding in CRIE. To qualify as a "major" allergen, an allergen must fulfill two independent criteria. At least 25% of the relevant patient sera must give strong IgE binding (CRIE classes A–D), and at least 50% of all the sera must show at least some specific IgE binding (CRIE classes A–F) (2). This definition is a slight modification of Løwenstein's definition of major allergens (4). To qualify as a "minor" allergen, less than 10% of the patient's sera must give rise to IgE binding in CRIE classes A–F. Allergens not belonging to any of these extreme categories are defined as "intermediate" allergens. The CRIE classes used in the definitions are obtained by employing a reference system in CRIE previously published (2). Any grading system in CRIE, however, which allows discrimination between strong and weak IgE binding may be used, e.g. the point system described by Løwenstein et al. (6).

Ag-32 is the only "major" allergen which has been detected in *C. herbarum* so far (1). In contrast to the potency of Ag-32 as an allergen, it seems to be a poor antigen in the rabbit IgG system. Only one out of 12 rabbits that have been immunized at least 1 year with extracts containing Ag-32, has responded with synthesis of precipitating antibodies to this allergen. Ag-32 has a molecular weight of approximately 13 000 daltons and consists of five molecular variants differing in isoelectric points from 3.4 to 4.4 in intervals of approximately 0.25 pH units (3). These variants were antigenically identical in the rabbit IgG system used since the immunoprecipitates fused without forming spurs. They were also allergenically identical in the human IgE system since no differences were observed in their ability to bind IgE from nine patient sera.

Ag-54 is an "intermediate" allergen according to the definition given above. Only 8/35 patients had specific IgE against Ag-54, but the radiostaining corresponding to this allergen was extremely dense (7/8 were in CRIE classes A or B) (1). Ag-54 had a molecular weight of approximately 25 000 daltons and an isoelectric point of 5.0 (3).

The degree of purity of the isolated allergens was expressed in terms of immunological criteria based on the use of available rabbit antisera and patient sera (3). The crude *C. herbarum* extracts gave rise to approx-

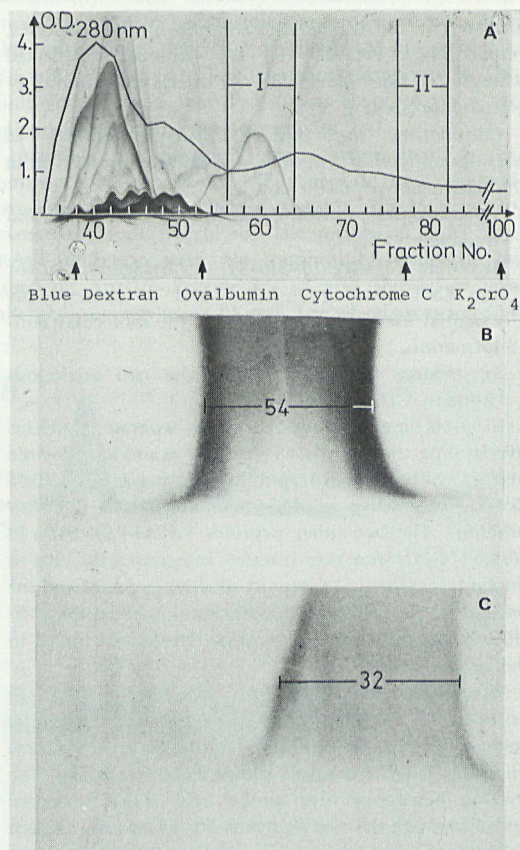


Fig. 1. Gel filtration of a *C. herbarum* extract on Aca 54 Ultrogel. (A) O.D._{280 nm} in the fractions and fused rocket immunoelectrophoresis using samples from selected fractions and anti-*C. herbarum*. The elution volumes of molecular weight markers in parallel experiments are indicated by arrows. (B) Autoradiography experiments using CRIE plates (above) incubated with a patient serum containing specific IgE antibodies only to Ag-32, followed by an incubation with ¹²⁵I-anti-IgE. (C) Autoradiographic experiments with serum from a patient containing high amounts of specific IgE antibodies only to the Ag-54 precipitate.

Table 1

Two different combinations of rabbit antisera and human sera which would both give radiostaining in CRIE corresponding to one precipitate (C) only. The (+) signs indicate the presence of antibodies specific for this particular allergen

Allergen	A	B	C	D	E	F
Rabbit antiserum I	+	+	+	+	+	+
Patient serum I			+			
Rabbit antiserum II	+	+	+			
Patient serum II			+		+	+

imately 60 precipitates, but the purified allergen preparations gave rise to only one precipitate each.

The final proof that the isolated allergens were actually Ag-32 and Ag-54, respectively, was obtained in two ways (3). Positive CRIE results were obtained when CIE plates containing an isolated allergen were incubated with a patient serum known to contain IgE only to the allergen in question. Negative CRIE results were obtained when CIE plates containing an isolated allergen were incubated with a pool of sera containing IgE to other important *C. herbarum* allergens, but not to the isolated allergen.

Distinct allergens in complex solutions may be monitored individually during fractionation by a combination of fused rocket immunoelectrophoresis and autoradiography techniques (3). An example of this is given in Fig. 1. Based on the initial CRIE experiments using the crude extracts, patient sera could be selected which contained IgE to only Ag-32 or Ag-54. Following a gel filtration experiment samples from the individual fractions were used to prepare fused rocket immunoelectrophoresis plates (Fig. 1a). Each plate was incubated with a selected patient serum followed by ^{125}I -anti-IgE and autoradiography as described for CRIE. The X-ray films from these experiments (Fig. 1b, c) showed radiostaining to only one precipitate which could thus be identified from the known IgE specificity of the patient serum used. This enabled demonstration of the exact elution pattern for the individual allergens. In contrast, it would be extremely difficult or impossible to obtain a similar degree of resolution using assay systems such as

RAST that measure only the total allergenic activity in each fraction. The limitation of the present approach is the availability of a patient serum which gives rise to radiostaining only to the allergen in question. If suitable patient sera are not available directly, it may still be possible to find a combination of a patient serum and a less broad-spectrum antiserum that gives radiostaining corresponding only to one precipitate (Table 1). If a sufficient degree of specificity is still not obtained, it is possible to combine the fused rocket immunoelectrophoresis with a series of crossed line immunoelectrophoresis experiments as described previously (5).

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