

COMMUNICATIONS

An Improved Procedure for Isolation of Ragweed Pollen Allergen Ra5

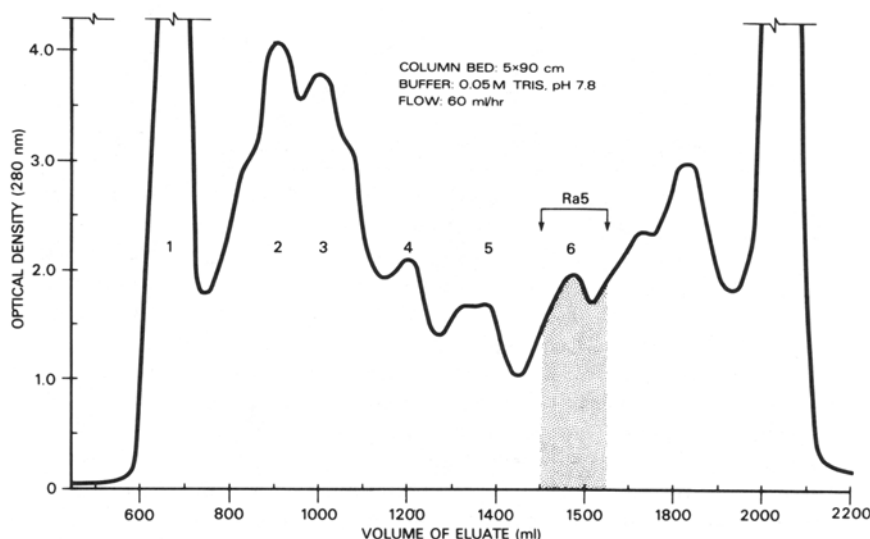
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Soon after the initial isolation of allergen Ra5 (1) from the pollen of short ragweed (*Ambrosia elatior*), human IgE-mediated sensitivity to this 5000 m.w. protein was shown to be significantly associated with the HL-A7 *creg* (cross-reacting group) of histocompatibility antigens (2, 3, 5). With determination of its amino acid sequence (6), Ra5 emerged as a potentially useful model for structure-function correlations in atopic hypersensitivity (4, 6, 7). However, extensive studies on the allergen have been hampered by the relatively low yields (approx. 10 mg/kg) obtained with existing isolation procedures (8) which have among other things utilized ether-extracted pollen as starting material. Following the finding by Marsh (3)

For partial depigmentation, WSR (150 ml) was passed through a column (2.5 x 30 cm) of DEAE-Sephadex A-25 (Pharmacia, Montreal), equilibrated and developed with 0.05 M Tris-HCl, pH 8.0 (*Tris*). The effluent (400 ml) was concentrated to 50 ml and sieved through a column (5 x 90 cm) of Bio-Gel P-10 (Calbiochem, La Jolla, Calif.), with *Tris* for development. Antigen Ra5 was reproducibly localized to the 6th major eluate peak (Fig. 1) by immunodiffusion analysis of the effluent by using specific rabbit or goat anti-Ra5 serum. A pool of peak fractions "6" obtained from four such column runs (pool equivalent to 400 g pollen) was concentrated to 25 ml and applied to a column (2.5 x 30 cm) of CM-Sephadex C-25

Figure 1. Filtration of WSR (after partial depigmentation) through Bio-Gel P-10 (100 to 200 mesh). Antigen Ra5 was localized by immunodiffusion analysis with rabbit and goat anti-Ra5 serum to peak fraction 6 (shaded area). Antigens E, Ra4 and Ra3 were similarly localized with appropriate antisera to peak fractions 1, 2, and 3, respectively (Roebber, M. and Goodfriend, L., unpublished observations).



that the aqueous extract formed from non-defatted pollen contained 330 to 350 mg antigen/kg, as determined by double antibody radioimmunoassay with anti-Ra5 serum, we restructured our method of isolation based on omission of the ether extraction step. As a result, we can report appreciable increase in yield of Ra5 in homogeneous form.

To prepare the aqueous extract, 200 g of short ragweed pollen (Sharp & Sharp, Everett, Wash.) were stirred in 1.6 liters of a solution consisting of 1 part of pH 8.4 borate-saline (6.2 g boric acid, 915 g sodium tetraborate, and 4.3 g NaCl) and 39 parts of 0.9 NaCl for 18 hr at 5°C. The extract was separated from the pollen by centrifugation in an IEC B-20 centrifuge (rotor 874) for 15 min at 10,000 rpm. The water soluble ragweed (WSR) was Millipore filtered and concentrated to 300 ml in a Diaflo ultrafiltration cell equipped with a UM-2 membrane (Amicon Corp., Cambridge, Mass.).

equilibrated and developed with 0.04 M sodium phosphate buffer, pH 6.8. Two well separated fractions were eluted (Fig. 2), the second of which contained almost pure Ra5. The Ra-5-containing peak fraction was recycled on the same ion-exchanger, concentrated to 25 ml, and applied to a column (2 x 90 cm) of Bio-Gel P-10 (equilibrated with *Tris*). Antigen Ra5 was eluted as a very sharp peak (Fig. 3) in yields of 40 to 45 mg/400 g pollen (based on $E_{280}^{1\text{cm}, 1\text{mg}} = 2.36$ (8)). Ultrapure Ra5 could be obtained, with only a slight reduction in yield, by cycling through a column (2.5 x 90 cm) of Bio-Gel P-4.

The allergen Ra5 preparation was homogeneous by acrylamide gel disc electrophoresis (Roebber, M. and Goodfriend, L., unpublished observations) and isoelectric focussing (4; Roebber, M. and Goodfriend, L., unpublished observations). Its amino acid composition agreed closely with published data for Ra5 isolated from ether-extracted pollen (Table I). Samples of

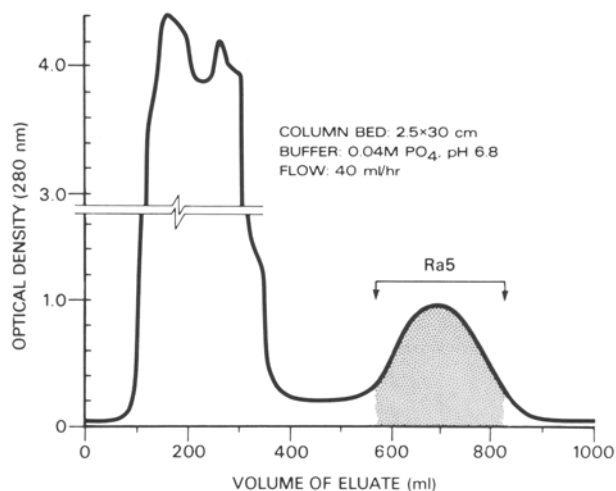


Figure 2. CM-Sephadex chromatography of a pool (equivalent to 400 g pollen) of peak fraction 6 (see Fig. 1).

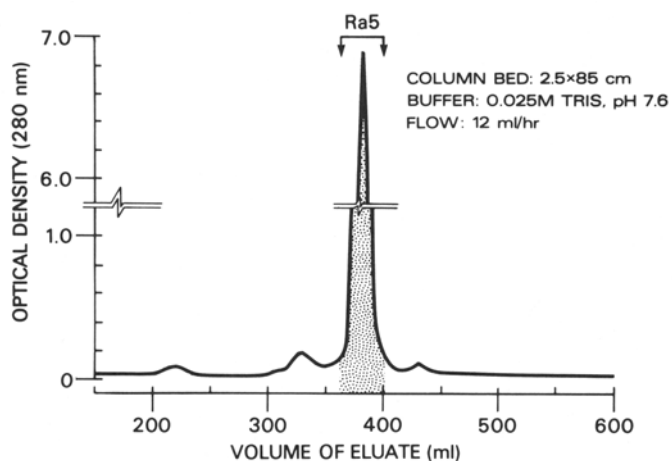


Figure 3. Filtration through Bio-Gel P-10 (200 to 400 mesh) of Ra5 containing CM-Sephadex peak fraction (see Fig. 2).

Ra5 prepared by previous and present methods showed lines of identity on immunodiffusion in agar with rabbit anti-Ra5 serum and gave essentially identical cutaneous reactions on direct skin test of ragweed-sensitive patients (Roebber, M. and Goodfriend, L., unpublished observations).

The present procedure provides a rapid method of preparing ultrapure Ra5 in yields of about 100 mg/kg pollen. It also permits the simultaneous isolation of other ragweed pollen allergens (E, Ra3, etc.) in substantial but not unusual yields (Roebber, M. and Goodfriend, L., unpublished observations). Although certain of the chromatographic steps previously employed (8) to isolate antigen Ra5 were modified in this study, it seems likely that the critical factor resulting in the 10-fold increase in yield of this allergen was omission of the conventional ether extraction of the pollen. It is not clear at present if the deleterious effect of ether extraction was due to concomitant protein denaturation (as has been suggested for antigen E by Goldfarb (9)), to solubilization of the low

TABLE I
Amino acid composition of allergen Ra5

	No. of Residues per Mole Ra5	
	Found ^a	Reported ^b
Lysine	3.8	3.7
Histidine	0	0
Arginine	2.4	1.9
Aspartic	1.7	1.8
Threonine	0	0
Serine	3.8	3.9
Glutamic	4.5	3.9
Proline	3.4	3.0
Glycine	4.0	3.7
Alanine	2.7	2.8
Cystine/2	6.7	5.9
Valine	3.4	3.2
Methionine	0	0
Isoleucine	0.9	0.9
Leucine	1.3	1.3
Tyrosine	2.8	2.8
Phenylalanine	0	0
Tryptophane	ND	1.8

^a The values were averaged from analysis of 48- and 72-hr acid hydrolysates (as determined in a Spinco 120B Amino Acid Analyzer), assuming 4 glycines per mole Ra5 (6).

^b From reference 8.

molecular weight Ra5 protein in the ether phase, or other unsuspected factors. While current studies are designed to discriminate among these possibilities and to determine the conditions under which ether extraction may be efficaciously employed for isolation of Ra5, the present procedure provides an alternative to ether extraction in the use of DEAE-Sephadex column chromatography (under appropriate buffer elution conditions) for partial depigmentation (and delipidation?) of the aqueous pollen extract. The resultant 10-fold higher yields of Ra5 should facilitate more extensive use of this protein for study of some basic problems of allergen structure and activity.

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