

ISOLATION OF THREE ALLERGENIC FRACTIONS OF
THE MAJOR ALLERGEN FROM OLEA EUROPEA POLLEN
AND N-TERMINAL AMINO ACID SEQUENCE

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A method to isolate the major allergen from olive pollen (Ole e I) in high yield is described. The allergenic fraction has been separated into 3 subfractions by reversephase HPLC. All these fractions were reactive to allergic sera from olive-sensitized patients, giving similar responses. No significant differences were observed between the amino acid compositions of these three proteins. The amino acid sequence of the first 27 amino acid residues from the N-terminal end is given. No homologies have been detected between Ole e I and other known allergens obtained from pollen. © 1990

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Plant pollens are one of the most important causes of allergy. The incidence of these allergens on human population is clearly related to the geographic distribution of the plants. Thus, the allergenicity to olive pollen is among the principal origins of the allergic diseases in mediterranean countries (1), this being one of the most significant demarcational allergens.

Scarce knowledge is available regarding the structure of pollen allergens. The best known are proteins isolated from Lolium and Ambrosia species, and many of them have been sequenced (2-8). Among those important local pollen allergens, only the complete amino acid sequence from that of white birch (Betula berrucosa) is known (9), and only partial sequence data have been published from others such as Japanese cedar, hazel, or alder. Many attempts have been done to identify the allergens from Olea europea pollen (10,11). Recently, Lauzurica et al., 1988 (12) have reported the isolation of

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Abbreviations: TFA, Trifluoroacetic acid; RP-HPLC, reverse- phase high pressure liquid chromatography; PITC, phenylisothiocyanate; EIA, enzyme-immunoassay.

Ole e I and Ole e II, the two major allergens from this common mediterranean tree. These molecules are proteins of 19 and 8 kDa, respectively.

Highly purified allergens are useful for clinical diagnosis and hyposensitization treatments, as well as to improve the knowledge on their immunological responses (13-15). Moreover, the comparison of the amino acid sequences of the pollen allergens is a requirement in understanding the molecular structure-allergenicity relationships.

We herein propose a new simple procedure to purify Ole e I, the main responsible for the allergenic activity of the olive pollen (12). The procedure described remarkably improves the purification yield and allows the separation of three allergenic subfractions. The isolated subfractions were automatically sequenced and the existence of microheterogeneity determined.

MATERIALS AND METHODS

Olive pollen was obtained from Allergon (Sweden). The crude extract was prepared by saline and ethyl-ether extractions (16). The obtained dried powder was dissolved in 0.25 M ammonium bicarbonate, pH 8.0, and loaded onto a Sephadex G-75 fine column (18 x 800 mm) equilibrated in the same buffer. The 19 kDa allergenic fractions were pooled and lyophilized. A reverse-phase HPLC step on a μ Bondapak C-18 column (7.9 x 300 mm) was used to purify this allergen, by using a Beckman system with an acetonitrile gradient in 0.1% TFA. The eluent was continuously monitored at both 214 and 280 nm wavelengths.

SDS-polyacrylamide gel electrophoresis was performed according to Laemmli (17). Alternatively, the samples were analysed after reduction of the potential disulphide bridges at 85 °C for 20 min, in the presence of 2-mercaptoethanol. Amino acid analyses were performed on a Durrum D-500 amino acid analyzer, after hydrolysis of the proteins, in the conditions previously described (18). Performic acid oxidized samples were used to determine half-cystine content. Reduction and radioalkylation of the allergen was carried out as described (19). N-terminal Edman degradations were carried out automatically by using an Applied Biosystems model 477A sequencer. The resulting phenylthiohydantoin-amino acid derivatives were identified by using a 120 A on-line PTH-analyzer and the standard Applied Biosystems program. The University of Wisconsin Genetic Computer Group (UWGCG) program and The Protein Sequence Data Bank from the Pasteur Institute were used for searching sequence homologies.

Enzyme-immunoassays were performed according to the method of Wide et al. (20) with minor modifications. Nitrocellulose discs were coated with either Olea europea crude extract or isolated fractions, at the same protein concentration, and incubated with a pool of allergic sera. All sera were screened with Phadebas RAST and showed specific IgE-binding (EIA-class 3-4). System was developed with anti human IgE- β galactosidase labelled (Phadezym RAST 60, Pharmacia, Uppsala, Sweden). For the EIA Inhibition assays, five-fold serial dilutions of Ole e I subfractions (257 μ g/ml) were incubated with a pool of allergic sera (1/2 dilutions). After 16 h of incubation EIA was performed as described above by using discs coated with Olea europea crude extract.

RESULTS AND DISCUSSION

An allergenic solution was obtained from the Olea europea pollen after salt-extraction with 0.25 M ammonium bicarbonate and further ethyl-ether

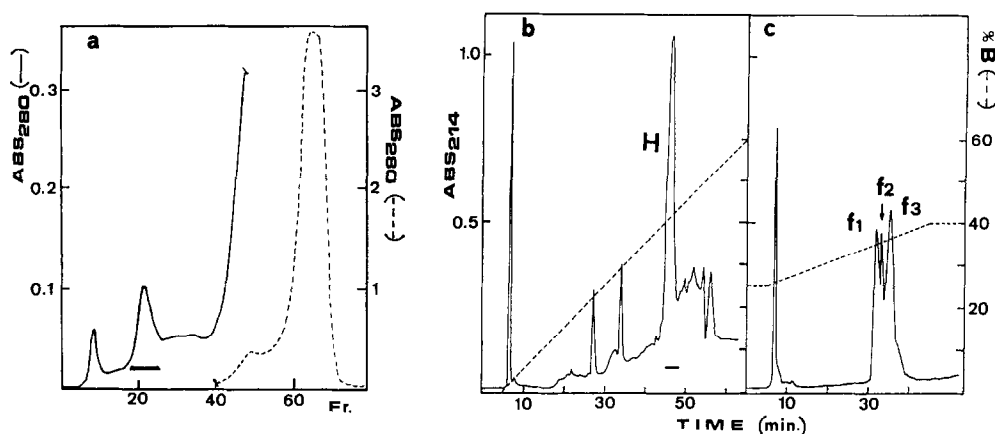


Figure 1. Chromatographic elution profiles obtained to isolate Ole e I. a) fractionation on Sephadex G-75 of the saline extract from olea pollen; fractions under the bar, containing the 19 kDa allergenic component, were pooled and lyophilized. b) RP-HPLC chromatography of collected fractions from (a) on a μ Bondapak column using a 0-60% gradient of buffer B (0.1% TFA in acetonitrile). c) RP-HPLC profile of the allergenic fraction H, but using a 25-40% gradient of buffer B.

extraction which removes most of the pigments and lipid material. The salt-soluble fraction was chromatographed on a Sephadex G-75 column (Fig. 1a). A large peak eluting at the total volume of the column contains salt soluble yellow pigments, with no significant protein content as deduced by amino acid analysis. Thus, the allergenic fractions appearing at about 19 kDa (fractions 19 to 26) were pooled and lyophilized. They represent the main polypeptidic component of the extracted material. The lyophilized fractions were further purified by reverse-phase HPLC (Fig. 1b) with a 0-60% acetonitrile gradient. The peak containing the allergenic activity accounts for the major olea allergen (Ole e I) previously described by Lauzurica et al. (12), according to their electrophoretic patterns. The typical electrophoretic behaviour of Ole e I, as a double band at 18 kDa and 20.5 kDa, has been attributed to the existence of two differently glycosylated forms (12). This is in agreement with our results, since the 20.5 kDa component was strongly dyed with the fucine reagent but not the 18 kDa form. On the other hand, the treatment with 2-mercaptoethanol at 80 °C for 20 min does not changes their SDS-electrophoretic mobilities.

This purification procedure yields 10-12 mg of the allergenic protein per gram of pollen powder, which stands for a remarkable recovery improvement. The allergenic fraction (H) was rechromatographed in the same RP-HPLC column above described but using a 25-40% acetonitrile gradient. In these conditions three discrete well defined subfractions were separated (Fig. 1c). All of them showed similar allergenic activities (EIA class 3-4) and electrophoretic patterns (Fig. 2). Nevertheless, the results obtained with EIA inhibition show a lower inhibitory capacity for subfraction f3 (data not shown).

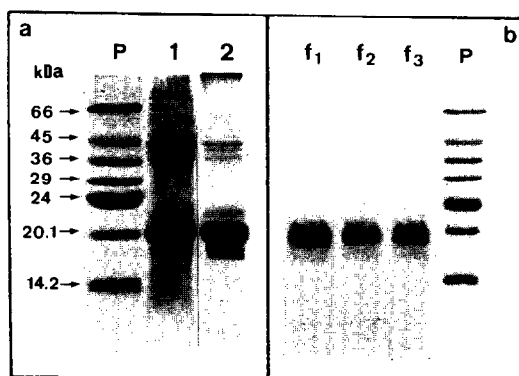


Figure 2. SDS-PAGE patterns of different steps during the purification of Ole e I. Molecular weight standard proteins (P). a) Salt-soluble protein extract from olive pollen (1); Allergenic fraction (19 kDa) from sephadex G-75 column (2). b) f1, f2, and f3 subfractions of Ole e I obtained from reverse-phase HPLC column.

The amino acid compositions of these three separated subfractions do not show significant differences (Table 1). This suggests the existence of isoallergens, as defined by Johnson and Marsh (21), for Ole e I. These three polypeptides have 5-6 half-cystines. Cys does not appear in the whole amino acid sequence of the birch allergen (9). This fact and the importance of cysteine residues in protein structure would exclude any homology between both plant allergens.

Table 1. Amino acid composition of the f1, f2 and f3 subfractions from Ole e I

	Molar %			X (%)	Ole e I**
	f1	f2	f3		
CYS*					5-6
ASX	10.2	9.7	10.0	10.0	16
THR	7.3	6.9	7.5	7.2	12
SER	5.1	6.0	5.4	5.5	9
GLX	12.8	12.4	12.8	12.7	20
PRO	10.7	11.4	11.3	11.1	18
GLY	8.0	8.4	7.5	8.0	13
ALA	4.1	4.3	3.8	4.1	7
VAL	5.3	5.5	5.6	5.5	9
MET	1.5	1.5	1.8	1.6	2-3
ILE	5.8	5.6	6.1	5.8	9
LEU	6.7	6.8	6.8	6.8	11
TYR	3.3	3.4	3.1	3.3	5
PHE	4.7	5.2	5.3	5.1	8
HIS	1.6	2.0	1.6	1.7	3
LYS	6.9	6.3	6.3	6.5	10
ARG	4.8	4.7	4.8	4.8	8
TRP					N.D.

* Determined as cysteic acid in the H fraction after 24h hydrolysis.

** Results are expressed as the nearest integer value based on 166 residues for the native protein, which would correspond to 19 kDa.

N.D. Not determined.

f1	E D	^V I	P Q P P V S Q F H I Q G Q V Y ? D T ? P A G F I ...
f2	E D	^V I	P Q P P V S Q F H I Q G Q V Y ? D T ? P A G F I ...
f3	E D	^V I	P Q P P V S Q F H I Q G Q V Y ? D T ? P A G F I ...
H	E D	^V I	P Q P P V S Q F H I Q G Q V Y C D T C P A G F I T ..

Figure 3. N-terminal amino acid sequences of the peak H and the three subfractions obtained from Ole e I. (?) stands for not identified residue.

The N-terminal amino acid sequence of the three subfractions have been determined (Fig. 3). A single sequence was obtained, and no differences between the three subfractions were observed on the identified residues. Two amino acids, Val (40%) and Ile (60%), appeared at third position in a very similar proportion in the three sequenced molecules. No residues were identified at positions 19 and 22. Reduction and [¹⁴C]-carboxyamidomethylation of fraction H were performed. The radioactivity incorporated was not the expected, and many attempts to improve the labelling were not successful. However, the N-terminal amino acid sequence of this radioalkylated fraction H was carried out up to 28 cycles. The data obtained were coincident with the individual sequences of the subfractions. Moreover, low but unequivocally increased radioactivity was determined at cycles 19 and 22, allowing to propose the existence of Cys at both positions on the Ole e I sequence. Neither fraction H nor f1-f3 subfractions show double sequence, in spite of their electrophoretic pattern. This would indicate that Ole e I is a single polypeptide chain protein, glycosylation being responsible for the observed microheterogeneity. On the other hand, differences on sequence behind the determined positions can not be excluded. These facts and/or conformational changes (22) could be the reason for the different pattern of EIA-inhibition reactivity found in subfraction 3. Chromatographic and electrophoretic multiplicity has also been described for the major allergen from Japanese cedar pollen, and identical sequences were obtained for the five separated subfractions (23).

The primary structure of Ole e I does not show homology with other sequenced pollen allergens nor with other known proteins (Protein sequence data bank).

The knowledge of the complete sequence of this allergen will be of great importance for further investigation on the structure-allergenicity relationships of this protein.

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