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N-Terminal Amino Acid Sequence Homologies of Group V Grass Pollen Allergens

Abstract

In an earlier study, we presented data regarding the immunoaffinity purification and N-terminal sequencing of a major pollen allergen from orchard/cockfoot grass (*Dactylis glomerata*), now identified as the group V allergen *Dac g V*. In this paper, we have extended our investigations to include group V allergens from other grass species. Our data confirm the presence of group V-restricted characteristic N-terminal amino acid sequences containing a high alanine and hydroxyproline (P') rather than proline (P) content, and based upon two conserved elements (ADAGY and TPA/TP'A).

The complexity of plant pollen allergens and their relative importance in the development of atopic disease is well documented [1], and many techniques have been applied to their characterisation. It has been demonstrated that pollen allergens from the same group from different grass species are immunochemically similar, as defined by monoclonal antibodies [2], and/or polyclonal human serum IgE [3]. The importance of group I allergens has been firmly established, and although the group V allergen from *Phleum pratense* (previously Ag 25) was described in 1978 [4], the importance of group V allergens has only recently been recognised [5, 6]. Given the IgE-binding prevalence of the group V allergens [6], the elucidation of sequences from different grass species is very important. Thus, the aim of this study was to attempt to define N-terminal sequence homologies from group V allergens from pollen of closely related grass species.

Extracts were made from pollen of five different grass species: *Lolium perenne* (*Lol p*), *Dactylis glomerata* (*Dac g*), *Festuca rubra* (*Fes r*), *Phleum pratense* (*Phl p*) and *Secale cereale* (*Sec c*). Purified group V allergens were then isolated from these crude aqueous extracts by immuno-affinity chromatography using murine MAb 4B1 [7]. The purified allergens were shown to be recognised by IgE in atopic serum samples (results not shown). The group V allergens were resolved by electrophoresis in a 12% polyacrylamide gel in the presence of sodium dodecyl sulphate under reducing conditions, using the method of Laemmli [8]. Protein bands were visualised by staining with 0.1% Coomassie blue R250, as shown in figure 1. Thus, *Phl p V* was resolved to a major 33 kD band, with a fainter band at 29-30 kD. *Sec c V* and *Fes r V* appear as single bands of 29 kD, although the latter appeared to contain a number of fainter bands both above and below the major band. Two

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Table 1. Grass pollen group V allergen N-terminal sequences, obtained by direct protein sequencing and nucleotide sequencing of cDNA clones, aligned for maximum homology

DG 3 [12]	G Y T H A T H A A A G G K A T T E E Q K L
<i>Dac g</i> V 29 kD	A D A G Y T P' A A A G G P' A A
<i>Dac g</i> V 27 kD	G Y A P' A A P' G T G P K A * P E Q K L
<i>Sec c</i> V 29 kD	G Y A P' A A P' G T Q P K A * P E Q K L
<i>Fes r</i> V 29 kD	A D A G Y A P' * K * K A E
<i>Phl p</i> V [6]	A D L G Y A P' A T P' A A P' G A G Y T P' A T A
<i>Phl p</i> V [13]	A N L G Y A H A T H A A H G A G Y T H A T H
<i>Phl p</i> V 33 kD	A D L G Y G P' A T P' A A P' '
<i>Lol p</i> Ib (IX) [10]	A D A G Y T P A A * * T P A T P A * T
<i>Lol p</i> Ib (rec) [10]	A D A G Y T P A A A A T P A T P A A I P A G G
<i>Lol p</i> V 28 kD	A Y P' P' Y P' P' A * P' P' P' * A A
<i>Poa p</i> IX (rec) [11]	A D V G Y G A P A T L A T P A T P A A P A A G

* = No assignment made.

major bands of 29 and 27 kD were obtained from *Dac g* V, while the *Lol p* V sample appeared to contain a single protein band of approximately 28 kD.

In addition, the group V allergens were electroblotted onto polyvinylene difluoride ImmobilonTM membranes for N-terminal microsequencing [9]. Transferred protein bands were stained as before, excised and sequenced on an Applied Biosystems 470 A gas-phase sequencer, by Dr. John Fox, Alta Bioscience, University of Birmingham, UK. The high degree of alanine residues and hydroxyproline residues was confirmed by repeated analyses, and the sequences, aligned for maximum homology with previously published group V allergen sequences are shown in table 1.

Our data clearly show the presence of two essentially conserved motifs, based on ADAGY and TPA or TP'A (table 1). These results are essentially confirmed by the findings of other researchers, and include the nucleotide sequencing of a complete cDNA clone from *L. perenne*, originally described as *Lol p* Ib [10]. This allergen has subsequently been renamed *Lol p* IX [Knox, pers. commun.], but the debate continues as to whether group V and group IX allergens are the same. Furthermore, the reported sequence of a cDNA clone encoding the group IX isoallergen from *Poa pratensis* [11], included in table 1, has a high degree of similarity to sequences obtained from the other grass species studied here.

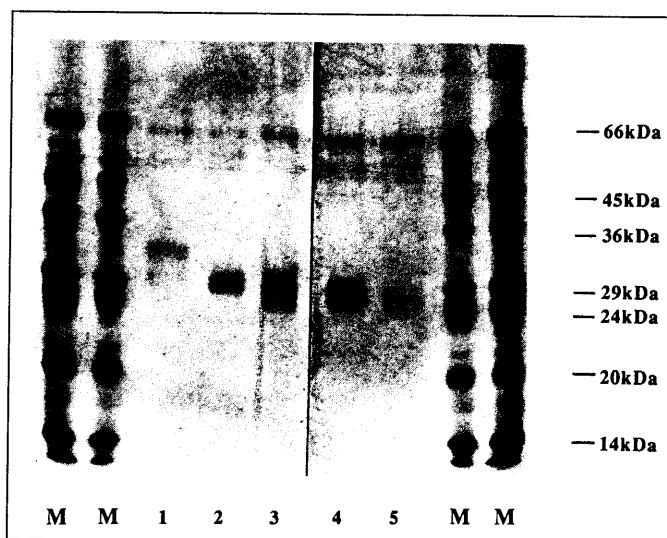


Fig. 1. = Electrophoretic separation (under reducing conditions) of 10–10 µg purified grass pollen allergens. Track 1: *Phl p* V; track 2: *Sec c* V; track 3: *Fes r* V; track 4: *Dac g* V; and track 5: *Lol p* V, on 12% polyacrylamide gels stained with 0.1% Coomassie blue R250. Positions of molecular weight standards are indicated.

In a previous report [12], and in common with Matthiesen et al. [13], we misassigned histidine residues to positions now shown to be occupied by hydroxyproline residues [5; this report] (table 1). This consistent misas-

signment, resulting from the difficulty in detecting the characteristically small twin hydroxyproline peaks by HPLC following microsequencing [5], was confirmed by repeated analyses using collagen as a control protein standard for hydroxyproline. It has been demonstrated that arabinogalactan proteins, which contain hydroxyproline and thought to be involved in plant cell adhesion [5, 14], are found in pollen extracts of *P. pratense* [15]. That these extracts proved to be allergenic might be evidence for arabinogalactan proteins as major allergens, although further data are required for confirmation. It is of interest to note that proline and not hydroxyproline is consistently detected in both *Sec c V* and *Dac g V* at similar distances from the N terminus, suggesting that the length of N-terminal peptide available for post-translational hydroxylation in the folded intact allergen varies amongst different grass species.

However, the data here do suggest evolutionary-conserved divergence of group V allergen sequences in a phylogenetically related manner, from an original pollen protein encoding gene. The significance of this conservation at the N terminus, particularly with reference to its function within the pollen grain is not known at this time. In addition, research by Singh et al. [10] has shown that the N-

terminal portion of the recombinant *Lol p IX* allergen was not reactive with human IgE. Rather, the C terminus appeared to be highly reactive with human IgE. This does not, however, exclude the possibility of IgE reactivity with N-terminal sequences or an antigenic effect engendered by proline hydroxylation.

It is important to note that the accuracy of N-terminal microsequencing may be suspect, reflected in the fact that although our *Lol p V* preparation contained the correct type of residues, they were incorrectly sequenced. This may reflect the presence of multiple forms of the allergen within a single resolved band. However, the combination of microsequencing of native allergens, together with the analysis of allergen-encoding cDNA clones, will be invaluable in the delineation of in vivo allergenic epitopes for immunotherapeutic targeting.

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