

EDITORIAL

The immunobiology of allergenic peptidases

Dust mites, bacteria and fungi produce a number of allergens demonstrating varying biochemical properties. Some are hydrolytic and non-hydrolytic enzymes, others are enzyme inhibitors and yet others are proteins involved in storage or transport. Of these diverse biochemical groups, proteolytic enzymes appear to be particularly potent allergens in susceptible individuals, and are often recognized by more than 90% of individuals allergic to the originating source. Such enzymatically active allergens are usually endopeptidases, cleaving protein substrates internally rather than at either the N- or C-terminal ends and most belong to the cysteine or serine peptidase families. They are usually secreted into the environment where they become aerosolized and inhaled. In mites, they are found in fecal pellets since they are involved in food digestion, and in fungi and bacteria, they appear to be involved in growth and/or spore germination (Table 1).

Interest in peptidase allergens derived from domestic rather than industrial sources was stimulated more than a decade ago when the major group 1 allergen from the house dust mite *Dermatophagoides pteronyssinus* (Der p 1) was shown to be a cysteine peptidase [1]. Similar peptidases were subsequently shown to be produced by other members of the clinically significant Pyroglyphidae family namely, *D. farinae* and *Euroglyphus maynei* [2, 3]. These 25 kDa acidic to neutral allergenic proteins are synthesized by cells lining the gastrointestinal tract as prepro-proteins comprising an 18 residue signal peptide, a propeptide of about 80 residues, and a mature protein of about 221 residues. The pyroglyphid mite-derived enzymes are structurally very similar (81–84% sequence identity), and each contains a potential glycosylation site at amino acid residue position 52/53 in the mature protein sequence. In this issue of the journal, Mora and colleagues [4] now report the first description of a group 1 cysteine peptidase allergen from the non-pyroglyphid mite *Blomia tropicalis* (Family: Glycyphagidae), a dust mite associated with allergic sensitization and asthma in tropical and subtropical regions. Their studies reveal some interesting similarities and differences between pyroglyphid and non-pyroglyphid cysteine peptidases with potentially significant consequences for our understanding of the immunobiology and evolution of the group 1 mite allergens.

In enzyme taxonomy, clans are formed by groups of enzyme families, members of which originate from a single ancestral enzyme but whose relationship is not apparent from a simple comparison of primary structure because of substantial sequence divergence during evolution [5]. Usually the clearest evidence of a clan relationship comes from highly conserved substructures, such as areas around catalytic sites or cysteine residues necessary for disulphide bond formation and structural integrity. In contrast, family and subfamily level relationships are more evident from primary sequence analyses, and a member of a family will show a significant relationship to other members present therein. Papain is the archetypal CA C1 cysteine peptidase, with the group 1 allergens from *Dermatophagoides* spp. and *E. maynei* clearly falling within the same

grouping. An analysis of the available C1 family sequences indicates that it comprises at least two major subfamilies based on the size of, and sequence homology within, the pro-region [6]; one subfamily containing enzymes such as cathepsin B, whilst the other comprising papain and the cathepsins O, H, L, K and S. On the basis that Der p 1 can be assigned to the latter, it seems reasonable to propose that Blo t 1 is an orthologue of Der p 1 and is the latest member of this subfamily, despite its pro-region sequence being unreported. Complete pro-region sequence data will confirm the N-terminus residue proposed by the authors and provide insight into whether active Blo t 1 is formed from a single chain (which we consider most likely) or, like other members of this family (e.g. cathepsins H, L), it is a dimer formed by disulphide-bonded 'heavy' and 'light' chains generated by an additional cleavage that occurs during activation of the proenzyme.

The authors cloned cDNA coding for the Blo t 1 enzyme using sequence data derived from the study of pyroglyphid group 1 cysteine peptidase allergens. They took advantage of the highly conserved sequence similarity of the mite proteins in those regions known to be involved in catalysis to design polymerase chain reaction (PCR) primers to amplify any *Blomia* sequences corresponding to a group 1 analogue present in a *B. tropicalis* cDNA library. They were successful in obtaining an internal sequence for Blo t group 1 allergen and these data were then used to design primers to obtain 5' and 3' sequences using rapid amplification of cDNA ends (RACE)-PCR. The N- and C-terminal sequences were then used to construct primers to amplify the full length cDNA, which was expressed in *Escherichia coli*.

The sequence data obtained showed that the *Blomia* enzyme was similar to the pyroglyphid cysteine peptidases in that the cDNA encoded a mature protein of 221 residues with an molecular mass of ~25 kDa, and that the residues presumed responsible for catalytic activity are cysteine²⁵⁽³⁴⁾ and histidine¹⁶⁹⁽¹⁷⁰⁾ (Der p 1 counterparts in parenthesis). However, there are notable differences between the sequences, as the authors clearly highlight. For example, asparagine¹⁹⁰ is the third residue of the catalytic triad in pyroglyphid group 1 allergens, but is replaced by threonine¹⁸⁹ in Blo t 1, a substitution not reported for any other cysteine peptidase and thus of interest if subsequently confirmed. Unusually, an even number of cysteine residues is present in Blo t 1, whereas most members of this enzyme clan contain an odd number, with all but the catalytic residue contributing to disulphide bridge formation. In Blo t 1, the role of cys⁶⁷ is therefore unclear.

In Der p 1, the side chain of glutamine²⁸ (analogous to gln¹⁹ in papain) is thought to stabilize the development of the oxyanion during catalysis and hydrogen bonds to serine¹⁹¹ (ser¹⁷⁶ in papain). By inference, in Blo t 1, the equivalents of these are gln¹⁹ and ser¹⁸⁹. Homology modelling of Der p 1 suggests that tryptophan¹⁹² (trp¹⁷⁷ in papain) is a key component of the active site wall of Der p 1 in the interaction with substrate [7]; the potential equivalent in Blo t 1 is trp¹⁹¹. Structure-function

Table 1. Biochemical characteristics of some important allergenic peptidases

Peptidase	Mol. wt (K)	Frequency of recognition (%)	Catalytic type	Trivial name	EC* Number	Family/ Subfamily	Clan
Arachnids							
House dust mites							
Group 1	25	> 90	Cysteine		3.4.22.2	C1A	CA
Group 3	25	> 90	Serine	Trypsin	3.4.21.4	S1A	PA
Group 6	25	61–65	Serine	Chymotrypsin	3.4.21.1	S1A	PA
Group 9	30	> 90	Serine		3.4.21.–	S1A	PA
Bacteria							
Recognition							
<i>B. subtilis</i>	27		Serine	Alcalase	3.4.21.62	S8A	SB
<i>B. licheniformis</i>	27		Serine	Esperase	3.4.21.62	S8A	SB
<i>B. licheniformis</i>	27		Serine	Savinase	3.4.21.62	S8A	SB
Insects							
Cockroach							
Bla g 2	36	60	Aspartate-like		3.4.21.–	A1	AA
Fungi – Alkaline peptidases							
<i>Aspergillus fumigatus</i>							
Asp f 13	34	71	Serine	Oryzin	3.4.21.63	S8A	SB
<i>A. flavus</i>							
Asp fl 13	34	> 70	Serine	Oryzin	3.4.21.63	S8A	SB
<i>Penicillium chrysogenum (notatum)</i>							
Pen ch 13	34	> 70	Serine	Oryzin	3.4.21.63	S8A	SB
<i>P. citrinum</i>							
Pen c 13	34	> 70	Serine	Oryzin	3.4.21.63	S8A	SB
<i>Trichophyton rubrum</i>							
Tri r 2	29	43	Serine	Oryzin	3.4.21.63	S8A	SB
Fungi – Vacuolar peptidases							
<i>A. fumigatus</i>							
Asp f 18	34	79	Serine	Cerevisin	3.4.21.63	S8A	SB
<i>A. niger</i>							
Asp n 18	34	100	Serine	Cerevisin	3.4.21.63	S8A	SB
<i>A. flavus</i>							
Asp fl 18	34	> 70	Serine	Cerevisin	3.4.21.63	S8A	SB
<i>P. citrinum</i>							
Pen c 18	39	> 70	Serine	Cerevisin	3.4.21.48	S8A	SB
<i>P. chrysogenum</i>							
Pen ch 18	39	82	Serine	Cerevisin	3.4.21.48	S8A	SB
<i>P. oxalicum</i>							
Pen o 18	39	> 70	Serine	Cerevisin	3.4.21.48	S8A	SB
Fungi – Dipeptidylpeptidases							
<i>T. rubrum</i>							
Tri r 4	80	83	Serine		3.4.19.1	S9	SC
<i>T. tonsurans</i>							
Tri t 4	61	83	Serine		3.4.19.1	S9	SC

*Enzyme Commission nomenclature.

studies of papain suggest that aspartic acid¹⁵⁸ influences catalytic activity and a potential equivalent for this exists in Blo t 1 at D¹⁶⁸. However, Der p 1 and Eur m 1 lack this residue (Der p 1 has a tyrosine substitution), suggesting that Blo t 1 should have a different substrate preference from its pyroglyphid orthologues. Thus, whilst the substrate binding pocket of Blo t 1 has much in common with Der p 1 it also shares some features with papain that demarcate it from the pyroglyphid allergens. Beyond the catalytic region the amount of similarity is less (c. 35%). This is consistent with the modest sequence identity between other *Blomia* allergens and their pyroglyphid

counterparts, with the notable exceptions of tropomyosin and paramyosin (Table 2). Finally, it is likely that polymorphic differences in Blo t 1 will emerge given data obtained with the pyroglyphid group 1 allergens [8]. Whilst these minor sequence differences are unlikely to influence enzyme function, they may influence its immunobiology.

In this regard, Mora and colleagues showed that the Blo t 1 enzyme is allergenic, providing the first formal demonstration that *B. tropicalis* contains a group 1 allergen. Previously, others have been unable to confirm its presence, although protein bands with the anticipated molecular weight on SDS-PAGE

Table 2. A summary of cloned *Blomia tropicalis* allergens

Allergen	Frequency of recognition (%)	Biochemical identity	Percent identity with similar proteins from other mite species	Reference
Blo t 1	62	Cysteine peptidase	35	[4]
Blo t 5	45–79	Function unknown	43–70	[24, 25]
Blo t 10	20–29	Tropomyosin	95	[26]
Blo t 11	52	Paramyosin	90	[27]
Blo t 12	50	Function unknown*	21	[28]
Blo t 13	11	Fatty acid binding protein	42	[29]

* Equivalent allergen from *D. pteronyssinus* or *D. farinae* has yet to be described. However, there is limited homology with C-terminal region of putative chitinase from *D. farinae* [30].

were reported with limited frequency (28–52%) [9, 10]. Mora and colleagues demonstrated that Blo t 1 was recognized by 62% of *B. tropicalis* allergic individuals, contrasting with > 90% frequencies reported for native group 1 allergens from pyroglyphid mites. Although similar to that seen with other *B. tropicalis* allergens (Table 2), and consistent with the frequencies obtained in immunoblotting studies, the true frequency may be higher because reactivities of recombinant proteins may be relatively low [11, 12]. These data suggest that further immunochemical and biochemical studies using native material are required. Mora and colleagues also demonstrate little immunological cross-reactivity between the group 1 allergens derived from *B. tropicalis* and *D. pteronyssinus*. These findings are consistent with previous data [9, 10] but Mora and colleagues now provide the scientific evidence to explain such observations.

In contrast to the sources described above, peptidase allergens from cockroach have yet to be described, although the Bla g 2 allergen associated with the digestive system of the German cockroach (*Blattella germanica*) shows significant homology with the aspartate proteinases. This allergen, however, does not appear to be proteolytically active and belongs to the family of pregnancy-associated proteins [13] whose function is unclear. It is known, however, that extracts prepared from cockroach species such as *Periplaneta americana* possess peptidases which may be allergenic. For example, Lake et al. [14] showed that whole body and fecally enriched extracts from *P. americana* contain both serine and cysteine peptidases, although the former type of peptidase was absent from the fecally-enriched extracts. Such activity may therefore reflect the known cathepsin-L-like peptidase associated with haemocytes and the vitellogenesis process [15, 16]. Similarly, Iraneta et al. [17] identified, but did not extensively characterize, six peptidase bands by gelatin zymography with apparent molecular weights of 24, 32, 34, 65, 78 and 90 kDa. The majority were optimally active at pH 6 and inhibitors indicated the presence of both serine and cysteine peptidases. The latter three gelatinolytic bands were immunoreactive with a human cockroach-sensitive antiserum, suggesting allergenicity. Finally, a digestive chymotrypsin has been described [18].

In this issue of the journal, Bhat and colleagues [19] report the ability of cockroach serine peptidases to augment IL-8 responses induced by treating airway epithelial cells with TNF- α . This finding is consistent with the effects of purified peptidases from mites and fungi, where others have shown biochemical modulation of both epithelial and immune function by allergenic peptidases [20, 21]. Bhat and colleagues studied the effects of crude extracts from cockroach and thus it will

be essential to confirm that the active principles responsible for the effects they report are present in cockroach-derived material that is likely to be inhaled. Interestingly, the authors report that the cockroach extracts act synergistically rather than by stimulating epithelial cells independently. This is similar to the synergistic effects of *D. pteronyssinus* extracts on IL-4- and IL-13-induced cytokine responses in airway epithelial cells, although in this study it was not established that the effect was peptidase-dependent [22]. The underlying mechanism(s) involved in either the cockroach or house dust mite model is unclear but may reflect either the additive effects of two different signal transduction pathways, alterations in transcription factor expression and activity, or both [23]. In other studies, synergistic cytokine responses have been reported with a variety of agents implicated, in some way, with the development of asthma. For example, lipopolysaccharide, histamine and peptidoglycan have all been shown to increase cytokine production in epithelial cells. Such findings, together with those reported elsewhere on the pro-inflammatory properties of clinically significant aeroallergens, indicate that the continued study of how the airway epithelium interacts with the environment is vital.

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