

REVIEW

On the potential significance of the enzymatic activity of mite allergens to immunogenicity. Clues to structure and function revealed by molecular characterization

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Nearly 20 years ago, Kjell Aas presented the allergy and immunology communities with the thought-provoking question 'What makes an allergen?' [1]. An answer to this question and a rational mechanism to account for why certain individuals become sensitized to allergens are essential to the understanding of the immediate hypersensitivity response, as well as to the design of better diagnostic tests and more effective therapeutic approaches. In the intervening years there has been exciting progress in understanding the nature of the T-cell receptor, in characterizing the molecular attributes important in antigen recognition and in the genetics of atopy. However, several problems remain unsolved, and a full answer to the scientific challenge laid down by Aas remains to be provided. The absence of complete structural information for several major allergens currently prevents a full analysis of their antigenic determinants and the possibility that there is a defective regulation of IgE responses in certain individuals also requires further investigation. Moreover, there is neither a definitive explanation of how airborne allergens gain access to immunocomponent cells, nor is the identity of the cells involved firmly established. However, data from recent research into the structural and functional aspects of house dust mite allergens have suggested a unifying mechanism by which presentation may occur, although work will be necessary to refine and substantiate this provocative hypothesis in order to assess its importance relative to other factors.

Aas postulated that non-immunological variables, such as tissue permeability, might account for some aspects of why sensitization occurs in some individuals but not others [1]. In the case of presentation to the lung, the healthy airway mucosa is a tight and highly-regulated barrier that offers

intrinsically high resistance to the passive penetration of putative allergens [2]. Although the functional permeability of the airway epithelium is increased in established asthma [3] and freeze-fracture replicas reveal abnormal tight junction morphology [4], there are no data to indicate whether this is a primary defect in the airways of certain individuals who are genetically predisposed to the disease, or whether the phenomenon is secondary to inflammation. If abnormal mucosal permeability is not a primary defect, then other explanations for sensitization are needed. In the past, ideas about allergen sensitization invoked the complicity of extrinsic factors, such as microbial or viral infection [5]. The inflammatory reactions that result from such infections are suspected of compromising the barrier properties of the airway epithelium, thereby enabling allergen presentation to occur. In support of this, inflammatory cells certainly do have the potential to increase epithelial permeability [6,7]. There is also evidence to indicate that infections may be important risk factors in the development of asthma [8]. However, recent research has added a new dimension to this issue by suggesting that certain allergens may themselves have a direct potential to penetrate epithelial barriers [9]. If correct, this action would be expected to potentiate risk factors such as infections, particularly if proteinases also act as virulence factors for bacteria, as has been recently suggested [10]. The possibility that certain allergens might penetrate epithelial barriers arises from two lines of evidence. Firstly, the discovery that certain occupational and domestic allergens express enzymatic activity [reviewed in 11], and secondly, the observation that such allergens can cause epithelial damage *in vitro* [9].

Physical, immunological and biochemical properties of mite allergens

Allergens from diverse sources have now been recognized

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as expressing enzymatic activity. Most information exists for those derived from house dust mites of the genus *Dermatophagoides* which represent an important source of domestic allergens. At least four of these allergens are proteinases. We shall begin by reviewing the characteristics and properties of the known house dust mite allergens.

Group 1

This is currently the best characterized group of mite allergenic enzymes. Whole or partial amino acid sequences have been obtained for allergens from *D. pteronyssinus*, *D. farinae* and also from *E. maynei* of the genus *Euroglyphus* (Der p 1, Der f 1 and Eur m 1, respectively). These findings have established that there is approximately 80% conservation of identity amongst these 25 kDa acidic-neutral proteins [12–14]. The mature proteins contain 222–223 amino acids, although they are initially synthesized in pre-pro forms which presumably undergo enzymatic processing [13]. Der p 1 was one of the first mite allergens to be recognized as having putative proteolytic activity [15]. Database searches indicate that it had approximately 30% sequence identity with papain, but this observation was given further significance by the fact that the matching was most noted at amino acid residues that are known to be associated with the active site of well-characterized cysteine proteinases (Table 1). The existence of sequence identity between the group 1 allergens and a class of enzymes for which detailed crystallographic data exist has permitted the computational modelling and refinement of a structure for Der p 1 (see later) [16]. In the absence of crystallographic data for any of the mite allergens in their native forms, this computational modelling has helped to visualize the epitopes and enzymatic activity of what is a particularly important allergen. Polymorphisms are known to exist in the Der p 1 sequence [17], which account for the heterogeneity of these enzymes as judged by isoelectric focusing. However, it is not known whether the polymorphisms might also account for the tentative suggestion that Der p 1 exhibits some serine proteinase activity [18]. Thus, in the absence of definitive kinetic studies, the view that Der p 1 is a multifunctional enzyme must be considered provisional.

Group 2

These comprise small (14 kDa) neutral to basic non-glycosylated proteins that contain three internal disulphide bridges which are essential for their immunogenicity [19–21]. Cloning of cDNA and protein sequencing strategies have been applied to obtain sequence information for Der p 2, Der f 2 and Lep d 2 (originally designated Lep d 1 [22–24]. The mature proteins appear to be derived from preproteins that have leader sequences of 11–17 residues. Although no

Table 1. Conservation of catalytic site residues in papain, actinidin and Der p 1

Enzyme	Sequence											
	19 ^a	22		26	64		69		133			
Papain	Q	C G S C W		N G G Y P W V								
Actinidin	Q	C G S C W		D G G Y I T A								
Der p 1	Q ^c	C G S C ^c W		H ^d G ^d D ^b T ^b I ^b P ^b I								
	157		160		175	177		205	207			
Papain	V X ^e D H A		N S W		S S F							
Actinidin	V X ^e D H A		N S W		M P S							
Der p 1	P ^b N Y H ^c A ^b		N ^c S W ^d		Y ^b P Y ^b							

^a Residue numbering based on papain sequence.

^b Non-polar side-chain binding pocket.

^c Catalytic site residues.

^d Walls of active site cleft.

^e Deleted residue site X.

sequence identity has yet been found with enzymes *per se*, some preliminary physicochemical and biochemical data suggest that the group II allergens share sequence homology with a putative human epididymal protein of unknown function.

Group 3

Along with the members of group 1, some of the earliest indicators that mite allergens possess enzymatic activity came from studies on the 25–30 kDa proteins that comprise group 3 [25]. Amino acid sequence homology searches and subsequent investigations of substrate specificity and inhibitor susceptibility indicated that group 3 allergens express trypsin-like serine proteinase activity [25,26]. Heterogeneity of their isoelectric points and the existence of sequence polymorphisms indicate that numerous isoforms of the group 3 allergens exist (see Table 2). Although Der p 3 appears to be a serine proteinase that exhibits 40–50% sequence identity with vertebrate and invertebrate trypsins, the absence of a polyaspartyl-lysyl activation peptide indicates that the zymogen form must be activated by an, as yet, uncharacterized mechanism that is different from that of vertebrate trypsins [25]. As with Der p 1, the most notable sequence identity between the mite allergen and known enzymes is in the catalytic site. This should facilitate modelling of the structures of some of the group 3 allergens using approaches similar to those already successfully employed for Der p 1.

Group 4

At the time of writing, only the N-terminus of this minor

Table 2. N-terminal sequences of *D. pteronyssinus* and *D. farinae* serine proteinase allergens

Proteinase allergen	Sequence																			
Mite trypsin																				
	1																			20
Der f 3	I	V	G	G	V	K	A	Q	A	G	D	X ^a	P	Y	Q	I	S	L	Q	S
Der f 3	I	V	G	G	V	K	A	L	A	G	D	X ^a	P	Y	E	I	S	L	E	V
Der f 3	I	V	G	G	V	K	A	K	A	G	D	X ^a	P	Y	Q	I	S	L	Q	S
Der p 3	I	V	G	G	E	K	A	L	A	G	E	X ^a	P	Y	Q	I	S	L ^b		
Der p 3	I	V	G	G	E	K	A	L	A	G	Q	X ^a	P	Y	Q	I	S	L	Q	S
Der p 3 cDNA	I	V	G	G	E	K	A	L	A	G	E	C	P	Y	Q	I	S	L	Q	S
Mite collagenolytic serine proteinase																				
Der p 9	I	V	G	G	S	N	A	S	P	G	D	A	V	Y	Q	I	A	L ^b		
Mite chymotrypsin																				
Der f 6	(A)	(V)	G	G	Q	D	A	D	L	A	E	A	P	F	Q	I	S	L	L	K
Der p 6	(A)	I	G	X ^a	Q	(P)	A	A	E	(A)	(E)	A	P	F	Q	I	(S)	L	M	K

X^a Identity not established.^b End of sequence.

Brackets indicate assumed identity.

class of 60 kDa allergens has been sequenced, but the physicochemical and biochemical data support the view that group 4 allergens are amylases [27]. The significance of this type of enzymatic activity to their behaviour as allergens is not known, but it is noteworthy that the frequency of reactivity of mite allergic individuals to this group of allergens is significantly lower than that for the cysteine and serine proteinase allergens [11].

Group 5

Group 5 allergens comprise small (14 kDa) proteins about which relatively little is known at present [28]. The allergen from *D. pteronyssinus* has been subjected to cDNA cloning and these data predict a protein of 148 residues, with 113 of these in the mature protein [28]. Because this yields a higher molecular mass than the native protein, it is possible that the cDNA clone also encodes a propeptide sequence. No evidence for enzymatic activity of this group currently exists.

Group 6

These comprise a group of 25 kDa serine proteinases with substrate specificities that are similar to vertebrate and invertebrate chymotrypsins [29]. Partial sequences of the allergens from *D. pteronyssinus* and *D. farinae* have been determined and these show some similarity with the characteristic N-terminal IVGG amino acid signature

that is found in mite allergens with trypsin-like activity (Table 2).

Group 7

The biochemical identity of the 11–29 kDa proteins that comprise the group 7 allergens is currently unknown. Although cDNA cloning has been applied to the allergen from both *D. pteronyssinus* and *D. farinae*, their biochemical identities remain unclear and no significant homologies have been found [30]. The wide range of molecular masses reported for Der p 7 reflect variable degrees of glycosylation associated with the N-glycosylation site embedded in the deduced amino acid sequence [30].

Group 8

Relatively little is known about the group 8 allergens, although molecular cloning studies indicate significant sequence identity with glutathione-S-transferase [31].

Group 9

Recently, a third serine proteinase, distinct from the groups 3 and 6 enzymes has been isolated from *D. pteronyssinus* [32]. This allergen, designated Der p 9, has a molecular mass of 24 kDa and shows 61% sequence identity with Der p 3 and Der p 6 and contains the characteristic mite serine proteinase N-terminal signature (Table 2). It was shown to be distinct

from the latter enzymes on the basis of its reactivity with various inhibitors and synthetic substrates. However, some immunological cross-reactivity with Der p 3 was observed. Der p 9 has a specificity profile similar to chymotrypsin and cathepsin-G like enzymes and was capable of degrading type III collagen [32].

Other mite allergens

Several other proteins have been identified as being associated as potential dust mite allergens, but these are not yet classified according to the WHO/IUIS system of nomenclature. The data have been obtained by a variety of means ranging from conventional protein purification to cDNA cloning. The proteins concerned include a homologue of Hcs 71 heat shock protein [33], a molecule with glucoamylase activity and a protein that shows some sequence identity with a bacteriolytic enzyme from *Listeria monocytogenes* [34].

The putative role of proteinases in the disruption of cohesion in the epithelial barrier: a new property of allergens?

Although not all mite allergens have yet been shown to possess enzymatic activity, it can be seen from the foregoing discussion that at least four of the major house dust mite allergens are proteolytically active. Thus, the question naturally arises as to whether this property contributes in any way to allergenicity. As one of the most significant of the domestic mite allergens, most published investigations have so far centred upon the cysteine proteinase Der p 1. In this section we will review investigations that suggest some exciting possibilities about how enzymatic activity might contribute to immunogenicity, and therefore the pathogenesis of atopic asthma.

One of the long-standing questions in understanding the process of sensitization to airborne allergens is what factors explain the high frequency with which certain molecules are associated with allergenic activity, whilst others are not. Individual susceptibility to sensitization is likely to be determined by a variety of factors, including genetic predisposition. However, not all foreign substances evoke an antigenic or allergic response, and even when they do the frequency of such reactivity may be dependent upon factors other than the gross amount of that particular substance in the environment. It seems reasonable to propose, therefore, that the process of antigen presentation is a critical determinant in the process of sensitization. Current thinking about this process in the airways suggests that interaction between antigen and dendritic antigen presenting cells is an essential component of the routine surveillance for potential antigens [reviewed in 35]. Antigenic determinants are then

believed to be processed and shuttled through the body of the antigen presenting cell towards T-lymphocytes present in lymph nodes some distance from the point of allergen entry.

In support of the view that dendritic cells are important in this role is the fact that their numbers increase in response to inflammatory challenge [35]. There is currently little definitive evidence regarding the spatial localization of the tips of the dendrites of these antigen presenting cells in the airway epithelium. Under normal circumstances it seems unlikely that they sample the luminal environment because dendritic projections beyond the apical aspect of the epithelium have yet to be reported. It is thus a reasonable assumption that the dendritic projections terminate intraepithelially and are, therefore, shielded from the external environment by columnar epithelial cells. Presentation of antigen would therefore require transcytosis of the protein or an increase in paracellular permeability of the epithelium. There is currently no credible evidence for the former, although the possible existence of such a mechanism would raise some interesting questions about the physiological function of the transporters that would be required, and the identity of their endogenous ligands.

Other mechanisms for antigen presentation may exist. One of these invokes the possible role of bronchus-associated lymphoid tissue (BALT). BALT consists of follicular aggregates of lymphocytes, dendritic cells, high endothelial venules and specialized epithelial cells that are characterized by extensive surface invaginations that are presumed to trap and process putative antigens [36,37]. However, BALT is not a constitutive element of the human respiratory tract even under the barrage of 'real-world' antigen exposure [38]. It can appear with a sparse distribution in some cases of certain diseases, suggesting that its presence may be only transient [39–41]. Such episodic expression might help protect the host from the inducing agent, but it might also increase the likelihood of unwanted reactions to other substances at particular periods. Whether BALT plays a role in allergic sensitization is not known but, by analogy with other specialized organ-specific lymphoid tissue, it would be expected to orchestrate IgA- rather than IgE-mediated responses [42].

Another possible mechanism for antigen presentation is that airway macrophages present antigen directly to luminal lymphocytes. Whilst attractive, this process would depend on the return of luminal lymphocytes to the lymphatic system. Although antigen-induced T cell influx into the airway has been demonstrated, no specific efflux pathways have yet been identified [43,44]. Similarly, sensitization might take place if macrophages efflux from the lumen into the airway mucosa and present antigen within the tissue. Irrespective of whether it is the lymphocytes or macrophages that leave the lumen, their efflux might be enhanced

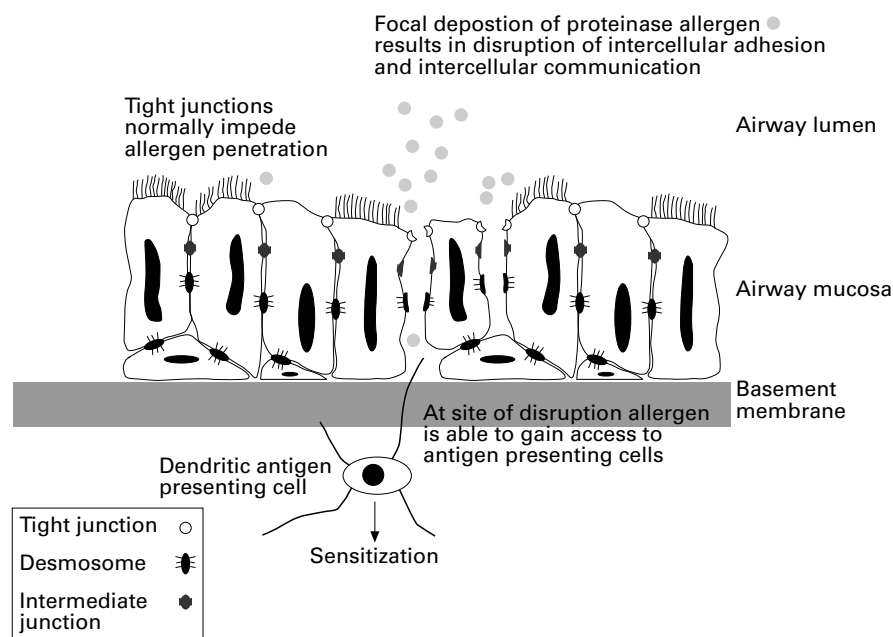


Fig. 1. Barriers to transepithelial penetration of macromolecular solutes and possible routes of entry for allergens with enzymatic activity.

by agents that compromise mucosal barriers. Thus, at least three potential mechanisms for antigen presentation rely, in part, upon increased tissue permeability.

The healthy tracheo-bronchial epithelium is a relatively tight barrier ($R = 200\text{--}700\ \Omega\text{cm}^{-2}$), consistent with its innate role in host defence. The high resistance is mainly due to tight junctions that limit the diffusion of solutes via paracellular pathways and other intercellular junctions which also provide structural integrity (Fig. 1). Although the transepithelial flux of allergens has yet to be visualized directly, proteins usually have low rates of transepithelial flux in the airway [45]. Thus, under normal circumstances, the healthy airway enjoys physical protection from tissue penetration by environmental allergens. Access to antigen presenting cells or the turnover of luminal T cell populations and macrophages is only likely to be increased when the integrity of the epithelial barrier is compromised (Fig. 1).

As reviewed in the introduction, it has been necessary in the past to invoke the complicity of bacterial or viral infections in the process of allergen sensitization [5]. Whilst semi-quantitative clinical evidence exists to support an apparent association between the prevalence of respiratory infections and the later onset of asthma, this does not constitute a formal, scientific proof of causality and other factors contributing to sensitization must be considered. The ability of allergenic proteins directly to facilitate their own transepithelial permeation and presentation to dendritic cells is therefore an exciting mechanism that would con-

tribute an insidious characteristic to these proteins [9]. Naturally, in the presence of other risk factors, such as infection, the penetration of allergens would be expected to be further augmented by inflammatory mechanisms mobilized in the host response. The production of some mediators might also be triggered directly in the vicinity of allergen deposition. For example, serine proteinases from house dust mites have been shown to activate the kallikrein-kinin pathways to produce proinflammatory and pain-producing kinins [46,47] in a similar manner to the activation of these pathways by bacterial proteinases [48].

Der p 1 and Der p 9 have been shown to mediate the release of interleukin (IL)-6, IL-8 and granulocyte-macrophage colony stimulating factor from bronchial epithelial cells (unpublished observations). Recent studies have also shown that Der p 1 cleaves the cell-associated 45 kDa CD23 low affinity IgE receptor from human B cell lines, releasing the stable 25 kDa degradation product sCD23 into culture supernatants [18,49]. It is thought that sCD23 is involved in stimulating the growth and differentiation of the precursors of plasma cells, T cells and basophils, as well as the autocrine regulation of IgE synthesis. This action of Der p 1 appears to be selective because other B cell markers (surface immunoglobulin, CD20, HLA-DR, HLA-DP, HLA-DQ, CD71 and CD49d) are not affected [18,49].

Enzymes from several proteolytic classes are known to cause disruption of epithelial cell interactions by non-cytotoxic mechanisms [2,6,7,9]. Histologically, such dis-

ruption is characterized by loss of lateral and basolateral adhesion between cells, suggesting that the enzymes disrupt morphoregulatory intercellular junctions [2,6,7,9]. The plausibility of proteolysis as a mechanism of epithelial disruption is obvious from the use of enzymes in the passage or dispersion of cells. This raises the question of what are the lines of evidence that adhesion molecules could be the targets of proteinases, in particular those enzymes that are important allergens. It has long been known that tryptic cleavage of the extracellular domains of cell junctions results in quite characteristic ultrastructural changes [50]. Electron-dense intercellular material is first degraded, resulting in physical separation of the junctional halves of adjacent cells. The separated halves then become internalized in cytoplasmic vacuoles [50].

The molecular components of these changes have been scrutinized as a means of probing the structure of desmosomes [51], but it has not hitherto been realized that this could also be the basis of a significant pathophysiological mechanism of tissue injury. The extracellular domains of desmocollins, desmogleins and other cadherins are sensitive to treatment with trypsin, but their susceptibility to other classes of proteinase is not yet characterized. It will therefore be especially important to investigate whether they can be degraded by environmental proteinases. It is noteworthy that proteolysis of adhesion molecules might also unmask a mechanism to perpetuate inflammatory cell infiltration because proteolytic fragments from desmocollins are putative chemoattractants [52].

We have recently shown that Der p 1 is not only a proteolytic enzyme that attacks synthetic substrates *in vitro* but that, over acute timescales, it can elicit an increase in the permeability of the bronchial mucosa to macromolecules such as serum albumin [9]. The effect of Der p 1, or cysteine-proteinase rich extracts from spent mite culture medium, can be inhibited by a matrix metalloproteinase inhibitor, suggesting that multiple catalytic pathways might be activated by mite proteinases [9]. Der p 1 also detaches cultured epithelial cell monolayers from both artificial and natural biopolymer substrata at similar mass concentrations or proteinase activities to trypsin or elastase from *Pseudomonas aeruginosa* [9]. These cell-detaching and permeability-enhancing actions of Der p 1 are absolutely dependent on its cysteine proteinase activity and can be prevented by using unreduced allergen proteinase or inhibited by the active site titrant E-64 [9]. Exposure of isolated bronchial segments to Der p 1 also results in disruption of epithelial architecture that can be visualized by even low resolution histological techniques [9] (Fig. 2). These effects were produced in the presence of dithiothreitol, confirming the need for Der p 1 to achieve catalytic competence as a cysteine proteinase to produce the effect. However, reduction of Der p 1 can also be produced by concentrations of glutathione that are found

in airway surface lining fluid [9,53], an observation that suggests a plausible mechanism for activation of this enzyme *in vivo*.

These changes clearly establish that the proteolytic activity of Der p 1 results in impaired adhesion in epithelial cells. The airway histology indicates that lateral cellular adhesion is more susceptible to disruption than is basal adhesion of cells to the biomatrix, although both are ultimately affected. In the absence of definitive studies to identify the precise intercellular junctions and adhesion molecules being attacked by Der p 1, it is impossible to determine whether the preferential loss of intercellular adhesion arises from spatial differences in the distribution of certain adhesion molecules or merely the extent of diffusional access to basally expressed sites of adhesion. However, such investigations are currently in progress and should provide important clues to the mechanism involved.

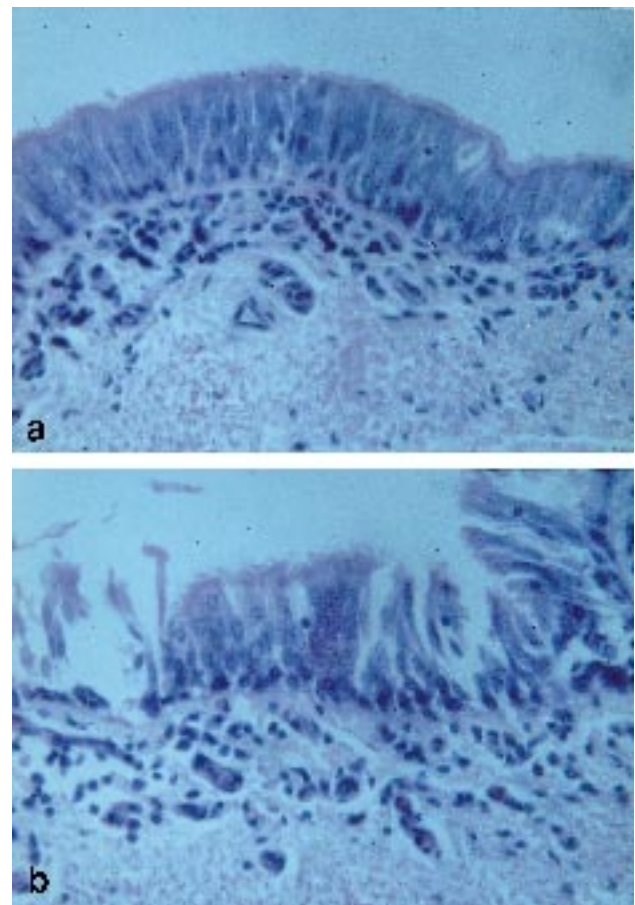


Fig. 2. Effect of exposure to control medium containing dithiothreitol (panel a) or reduced Der p 1 (panel b) on segments of bovine bronchial mucosa. Exposures were performed for 5 h using 230 Azocoll units/ml of proteinase activity. Airway segments were then formalin fixed and transverse sections prepared for microscopy.

The importance of allergen structure modelling in understanding their mechanism of action and their control

As a pre-requisite to appreciating the role of enzyme activity in inducing an allergic response, it is important to understand the mechanism of action in terms of substrate specificity and kinetic characteristics. These studies will be crucial in exploring the biological role of enzyme activity, and should also assist in the design of specific inhibitors for possible therapeutic applications. Crystal structures for common domestic allergens have yet to be reported. Although the production of recombinant allergens might yield high quality materials with which to undertake such work, it will be essential to demonstrate that recombinant proteins exhibit native biological properties before this type of structure-function study provides convincing data. Fortunately, the existence of sequence homology between certain proteinase allergens and enzymes for which excellent structural data are available, permits the

application of careful computational modelling strategies. At present, most data exist for Der p 1, but the successful application of similar strategies to serine proteinase allergens is anticipated.

On the basis of homology and modelling studies, Der p 1 is undoubtedly a member of the cysteine proteinase family (15,16). The model suggests that the molecule consists of two domains with a deep cleft between them (Fig. 3), and this structure is similar to papain, the archetypal member of the cysteine proteinase family. The amino acid residues of Der p 1 and related cysteine proteinases from which the structure of the molecule was modelled are shown in Fig. 4. The thiolate/imidazolium ion pair $ES^- \cdot HIm^+$ of the catalytic site (Fig. 5) is the dominant form of the active enzyme and consists of C34 and H170 (single letter amino acid code and Der p 1 numbering system). The corresponding residues in papain are C25 and H159. In cysteine proteinases, catalysis involves acylation. In papain, the interaction of the S^- ion of C25 with substrate generates a tetrahedral intermediate. The imidazole side chain of H159

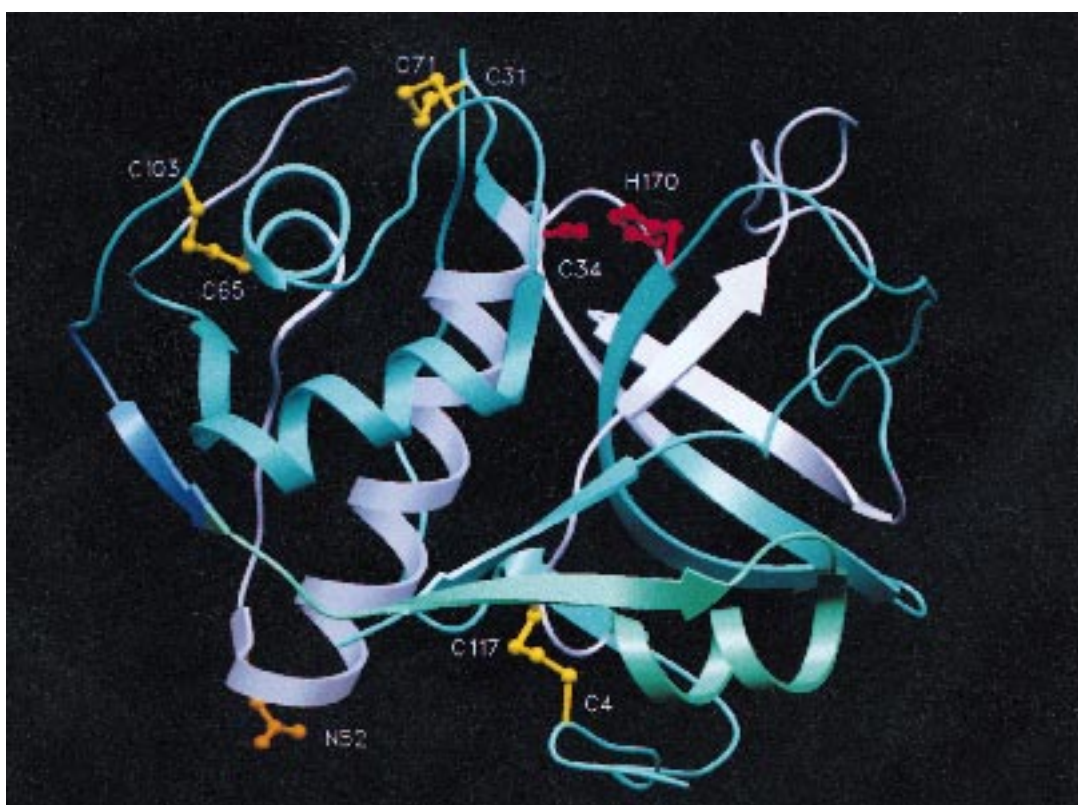


Fig. 3. The structure of Der p 1 produced by computational modelling. Der p 1 β -strands are indicated by arrows and α -helices as coils. Side chains of the three disulphides (yellow), the C34/H170 ion pair (red) and the glycosylation site (N52, orange) are shown explicitly. Human B-cell epitopes (residue ranges 1–33, 60–94, 101–111, 155–187 and 209–222 are coloured light blue and violet, residues 107–111). Overlapping T-cell epitope sites (residue range 107–131) are coloured green and violet (spanning the B-cell epitope site region). The figure was constructed using SETOR and is reproduced from reference 16 by kind permission of Oxford University Press.

9pap	i p e y V <u>d</u> w <u>r</u> q̄ k g A V <u>T</u> p V <u>k</u> n q̄ g s C g <u>S</u> <u>C</u> W A F <u>S</u> A V v T I <u>E</u> G I i k i r
2act	l p s <u>y</u> v <u>d</u> w <u>r</u> s a g A V v d I <u>k</u> s Q̄ g e C g G <u>C</u> W A F <u>S</u> A I a T V <u>E</u> G i N k i t
1ppo	l p e n V <u>d</u> w <u>r</u> k k g A V <u>T</u> p V <u>r</u> h q̄ g s C g <u>S</u> c̄ W A F <u>S</u> A V a T V <u>E</u> G I n k i r
1dpi	10 20 30 40 50 t n̄ a c̄ s i n̄ g n̄ a p a e i <u>D</u> L <u>r</u> q m r t V <u>t</u> p I <u>r</u> m q̄ g g C g <u>S</u> c̄ W A f <u>S</u> G V A A T <u>E</u> <u>S</u> a y l a h ββ φ φ αααααααααααααααα ••••• • • • • • • • • • G ! Y
9pap	t g n l n̄ q̄ Ȳ <u>S</u> <u>E</u> Q̄ <u>E</u> L L <u>D</u> c̄ <u>D</u> r̄ r̄ - - <u>S</u> - ȳ G c̄ n g G ȳ p w <u>S</u> A L q̄ l V a q y - G I <u>H</u> ȳ r̄ n t Ȳ p
2act	s g s l i s L <u>S</u> <u>E</u> Q̄ <u>E</u> L I <u>D</u> C g r̄ t q n̄ T̄ - r̄ G c̄ d g G ȳ l t d̄ G F q̄ f l i n̄ d g G I <u>N</u> t̄ e e n̄ Ȳ p
1ppo	t g k l v e L <u>S</u> <u>E</u> Q̄ <u>E</u> L V <u>D</u> C̄ <u>E</u> r̄ r̄ - - <u>S</u> - h̄ G c̄ k g G ȳ p p y A L ē ȳ V a k n - G I <u>h</u> l̄ r̄ s k̄ Ȳ p
1dpi	60 A B C D 70 80 A 90 r n q s L d̄ L A <u>E</u> Q̄ <u>E</u> L V <u>D</u> C̄ a - - - <u>S</u> q̄ h̄ G c̄ h g d̄ t I P r̄ G I ē Ȳ I q̄ h n - g V V Q̄ ē s ȳ Ȳ r̄ αφ αααααααα φ αααααααααα φ ••••• • • • •• • ••• • S K
9pap	ȳ ē g v q̄ r̄ y c̄ ī <u>S</u> r̄ ē k g p ȳ a a k̄ T d̄ g v r q v q̄ p ȳ n̄ q̄ g a L l y s I A n̄ q̄ P V <u>S</u> V v L Q A a
2act	ȳ t̄ a q̄ d̄ g d̄ c̄ d v a l q̄ d̄ q̄ k̄ ȳ V t I d t y e n V p ȳ n̄ <u>E</u> w a L q̄ t a V l̄ y q̄ P V <u>S</u> V a L d̄ A a
1ppo	ȳ k a k q̄ g t c̄ r̄ A k q v g g p i v k̄ T s g v g r v q p n̄ <u>E</u> g n̄ L l n a I A k̄ Q P V <u>S</u> V v V <u>E</u> s̄ k̄
1dpi	100 110 A B 120 130 A 140 ȳ v a r̄ ē q̄ s c̄ ī r p n̄ A q̄ r̄ - - F g I s m̄ ȳ c̄ q̄ l̄ ȳ p p n a n k̄ l̄ r̄ ē A L A - q̄ T̄ H̄ <u>S</u> A I A v l̄ i φ ββββ φ αααααααααα ββββββ • • • • • • • • V T
9pap	g - k d F q l y r g g - - - i f v G p̄ c̄ g n k v d̄ - <u>H</u> A V A A V G Y G p - - - n y I L I K̄ N̄ s̄ w
2act	g - d a F k q y a s g - - - i f t G p̄ c̄ g t a v d̄ - <u>H</u> A I V I V G Ȳ G t̄ ē g g v d̄ ȳ W I V K̄ N̄ s̄ w
1ppo	g - r p F q l̄ Ȳ k̄ g g - - - i f ē G p̄ c̄ g t k v d̄ - <u>H</u> A V T A V G Ȳ G k̄ s g g k̄ g y l l I K̄ N̄ s̄ w
1dpi	150 160 170 180 190 g i k d L d̄ a F r̄ h̄ y d ḡ ī t I I q̄ r̄ d n g y q p n̄ ȳ <u>H</u> A v <u>N</u> I V G Ȳ s̄ n̄ a q g v d̄ ȳ W I V <u>R</u> N̄ S̄ w̄ αααα φ ββ φω ββββββββββ φ βββββββ • • • • • • • • !

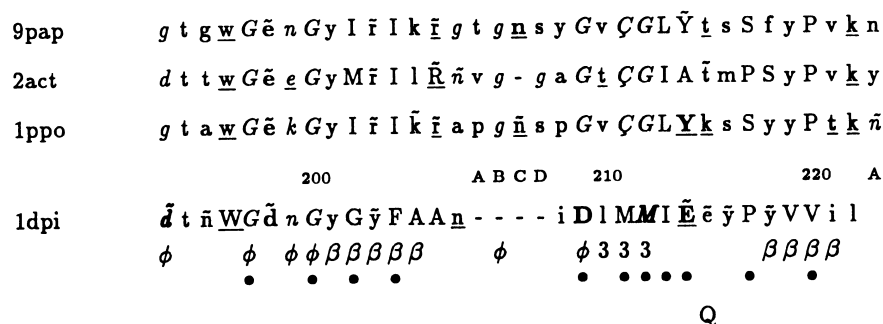


Fig. 4. Alignment of the sequences of cysteine proteinases of known tertiary structure and Der p 1. The structural environment at every residue position is represented using the program JōY (56). Structural environments at the residues of Der p 1 correspond to the modelled structure (56). Der p 1 numbering is shown. Conserved secondary structural elements are indicated. The isoforms of Der p 1 are indicated in the last row. The ! corresponds to the active site residues. Key to proteins: 9pap (papain); 2act (actinidin); 1ppo (papaya proteinase Ω); 1dpi (Der p 1). Key to JōY alignments: solvent inaccessible, upper case X; solvent accessible, lower case x; positive Φ , italic x; *cis*-peptide, breve $\checkmark$; hydrogen bond to other sidechain, tilde $\sim$; hydrogen bond to mainchain amide, **bold x**; hydrogen bond to mainchain carbonyl, underline x; disulphide bond, cedilla x^{c} .

is considered to play a number of roles involving maintenance of the thiolate anion in neutral and acidic media, and orientation of the side chain is mediated by hydrogen bonding with a conserved N175 residue in papain (N190 in Der p 1) which is the third critical residue of the catalytic triad.

A prominent feature of the active site wall of Der p 1 is the side chain of W192 which covers the H-N hydrogen bond. This residue is also conserved in papain (W177), where it is assumed to be in close contact with Schecter-Berger P₁' subsite non-polar residues in the binary complex with the substrate. This side chain of Q28 (Q19 in papain) probably helps to stabilize the oxyanion as it develops during catalysis. The Q28 residue is hydrogen bonded to S191 (S176 in papain). The NH₂ group of Q28 projects upwards into the cleft, with one hydrogen bond able to interact with the main chain carbonyl of C31, and the other free to interact with the substrate. Papain, in common with other cysteine proteinases, has a D158 residue which influences catalytic activity, but which is absent in Der p 1. However, Y169 in Der p 1 may be the counterpart of this residue. The side chain of Y169 intrudes into the Schecter-Berger S₂ substrate binding pocket and appears to be unique to Der p 1. When obtained, the crystal structure of Der p 1 should help to clarify the role of this residue in determining substrate specificity. At present there is little mechanistic information on the enzyme activity of Der p 1. The use of synthetic substrates, from which it is possible to obtain reliable kinetic data, will be invaluable in assessing ideas about specificity and inhibitors. We are currently exploring the role of specific substrates and time-dependent inhibitors to address some of the questions of structure-function.

As indicated earlier, Der p 1 is polymorphic. A number of naturally occurring variants have been identified; they

include A 3 G, H 50 Y, H 72 S, E 81 K, A 124 V, S 136 T and E 215 Q (17,54). The mutations occur mainly on surface-exposed residues and, on the basis of the model, they are unlikely to account for functional deficits (unpublished observations).

We have undertaken an analysis of the B-cell and T-cell epitopes of Der p 1 (55). A majority of the epitopes are abundant in positively charged residues (Table 3) and as a consequence have high isoelectric points, suggesting that the receptor regions of these epitopes are likely to be acidic. These observations contrast with those of the full length sequence of mature Der p 1 which has a theoretical PI value of 6.15 and a net charge of -3.65 at pH7.0. These observations may be relevant in designing synthetic peptides for potential use in therapy.

The expression of active Der p 1 and the construction of genetically engineered mutants which abolish or reduce enzymatic activity, should clarify the role of proteolytic activity in allergic reactions. For example, it should be possible to replace either the active site C with S or A, or replace the active site H residue with F, Y and T with no predictable effect on the structure of the protein, and to investigate whether proteolytic activity is crucial in

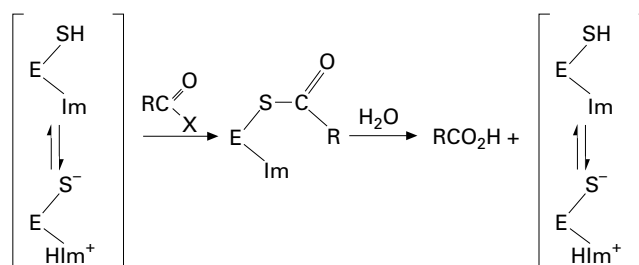


Fig. 5. General mechanism of action for cysteine proteinase.

Table 3. Peptide fragments corresponding to B cell and T cell epitopes of Der p 1

Peptide fragments (residue numbers)	T or B cell epitope	Net charge at pH 7.0	Isoelectric point pI
1–33	B	0.88	8.08
5–30	B	0.98	10.38
60–94	B	–3.39	4.74
101–111	B	2.93	12.20
111–139	T	1.17	8.43
118–136	T	1.22	9.55
155–187*	B	1.21	9.50
209–222	B	–3.02	3.47

* Terminal four residues removed from published sequence for epitope.

mediating an IgE response in cell culture and whole animal systems. Other possibilities include the design and synthesis of novel inhibitors which may abolish activity. If these approaches prove interesting, then an obvious move would be to adopt similar strategies for other dust mite proteinase allergens.

Conclusions

The finding that major domestic environmental allergens express functional enzymatic activity adds a new dimension to understanding how these molecules may be recognized by the immune system. In due course, the lines of investigation applied to Der p 1 will need to be extended to other house dust mite allergens in order to provide further information about the possible relevance of the concepts reviewed in this article. However, it is important to appreciate that not all allergens need be enzymes for the proposed mechanisms to be of significance because it would be expected that enzymatically active allergens might facilitate the presentation of those that were enzymatically inactive. The efficacy with which enzymes act as potential allergens is already established by the cases of respiratory allergies which arise from occupational exposure to such agents [57,58]. Further work is needed to establish exactly what mechanisms link enzymatic activity and allergic sensitization and provide at least some of the answers to the fundamental question posed by Kjell Aas. As highlighted in this review, significant progress has been made with the house dust mite allergens, particularly Der p 1. It will now be interesting to establish which other common allergens are enzymes and which are not.

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