

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/5630805>

# Cockroach Allergens: Function, Structure and Allergenicity

Article in *Protein and Peptide Letters* · February 2007

DOI: 10.2174/092986607782541178 · Source: PubMed

## CITATIONS

38

## READS

670

5 authors, including:



**Anna Pomés**

Indoor Biotechnologies Inc

153 PUBLICATIONS 3,218 CITATIONS

[SEE PROFILE](#)



**Sabina Wünschmann**

Indoor Biotechnologies Inc

58 PUBLICATIONS 1,489 CITATIONS

[SEE PROFILE](#)



**James Hindley**

Indoor Biotechnologies Inc

40 PUBLICATIONS 356 CITATIONS

[SEE PROFILE](#)



**Lisa D Vailes**

Indoor Biotechnologies Inc

105 PUBLICATIONS 3,092 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Antigenic determinants of asthma-associated allergens [View project](#)



The Role of Tregs in anti-tumour immunity [View project](#)

# Cockroach Allergens: Function, Structure and Allergenicity

A. Pomés\*, S. Wünschmann, J. Hindley, L.D. Vailes and M.D. Chapman

**FINAL**

INDOOR Biotechnologies, Inc., 1216 Harris Street, Charlottesville, VA 22903, USA

**Abstract:** Cockroach allergy is a widespread health problem in the world, associated with the development of asthma. The German and American cockroach species are important producers of a wide variety of allergens. Knowledge of their structure and function contributes to understand their role in allergy and to design tools for diagnosis and immunotherapy.

**Keywords:** Cockroach, allergens, asthma, molecular biology, molecular structure, function.

## HEALTH IMPACT OF COCKROACH ALLERGY

Cockroach allergy was first reported in 1964 by Bernton and Brown who described sensitization to cockroach in 44% of 755 allergy clinic patients in New York [1]. Since then, several studies have demonstrated the association between cockroach allergy and asthma, especially among patients with low socio-economic status, living in cockroach infested homes. Inhalation of cockroach extract by sensitized asthmatic patients elicited early, late-phase and bronchoconstriction responses [2-4]. Asthma is a major public health problem affecting 17 million people in the U.S. alone (6.9% of children under 18). Sensitization to cockroach allergens, as well as to other indoor allergens (cat, dog, dust mite, mold), is a major risk factor for emergency room admissions with asthma and for the development of asthma [5-11].

Exposure to cockroach allergens takes place by inhalation of particles suspended in the air. Lehrer and collaborators demonstrated the importance of cockroach feces, as well as the whole body of the cockroach, as sensitizing agents in atopic asthma [12]. The aerodynamic size of particles carrying cockroach allergens (and mite allergens), is larger (10-40  $\mu\text{m}$  of diameter) than the particle size associated with sensitization to cat or dog allergens (5-10  $\mu\text{m}$  diameter) [5]. Although disturbance of dust particles is required for exposure to cockroach allergens to take place, small amounts (1-10  $\mu\text{g}/\text{year}$ ) are sufficient to induce sensitization.

Cockroach allergens, Bla g 1 and Bla g 2, are used as markers of cockroach allergen exposure in environmental studies. The domestic reservoir concentration of mite and cockroach, but not cat, allergens has been reported to be closely related to the prevalence of sensitization in atopic children [13]. Huss and coworkers also demonstrated that levels of exposure measured by house dust analysis are important determinants of sensitization for dust mite and cockroach, but this relationship was not demonstrable for cat, dog or mold allergens [9]. Bla g 1 levels of greater than 8 U/g dust in children's bedrooms were strongly associated with increased hospital admission and other parameters of asthma-related morbidity [14]. There was a clear relationship

between current exposure to Bla g 1 and sensitization to cockroach allergen in asthmatic children 4 to 9 years old living in inner-city Baltimore [15]. The best correlation between sensitization and allergen concentration was found in the bedroom, even though the highest levels of cockroach allergens were in the kitchen. Prevalence of sensitization measured by positive skin test response to cockroach allergens increased from 32% among children exposed to 1-2 U/g dust to a plateau at 40-44% among those exposed to more than 4 U/g dust of Bla g 1 [15]. Similar results have been reported for Bla g 2 in case control studies of U.S. school children. A four-fold increase in the median Bla g 2 concentration resulted in a significant increase in the numbers of children who became sensitized [13]. From these studies, the threshold levels of cockroach exposure above which susceptible individuals would be at increased risk for sensitization or asthma symptoms have been defined as 2 U/g and 8 U/g dust of allergen, respectively. Avoidance studies have shown that standard housecleaning is partially effective in removing residual allergen over 8 months [16]. Reduction in cockroach allergen concentrations achieved through the combined intervention of occupant education, insecticide application and professional cleaning can be maintained with combined cockroach control [17]. However, re-infestation after abatement measures have disappeared makes this approach impractical in the long term. In order to prevent or improve cockroach allergy, the mechanisms involved in the development of cockroach allergy should be better understood.

## COCKROACH ALLERGY WORLDWIDE

Out of about 4000 described species of cockroaches, only four are ubiquitous and present in any human setting (*Blattella germanica*, *Supella longipalpa*, *Periplaneta americana* and *Blatta orientalis*) [reviewed in 18]. The most common domestic cockroaches associated with allergy are the German cockroach *Blattella germanica* in the US and Europe, and the American cockroach *Periplaneta americana* which is very common in South America and some Asian countries. Other cockroach species have been reported in homes. For example, the smokybrown cockroach (*Periplaneta fuliginosa*) has become an increasingly important peridomestic pest throughout much of the southeastern United States, Japan and southeast Asia [19]. This is the most predominant cockroach species in homes in Tokyo [20]. *B. germanica* is

\*Address correspondence to this author at the INDOOR Biotechnologies, Inc., 1216 Harris Street, Charlottesville, VA 22903, USA; Tel: 434 984 2304; Fax: 434 984 2709; E-mail: apomes@inbio.com

the dominant cockroach species in residences of Busan and Seoul in the Republic of Korea, followed by *Periplaneta fuliginosa* and *Periplaneta americana* [21]. In the Bangkok metropolitan area (Thailand), the following cockroach species have been found in sixty households of allergic patients: *Periplaneta americana* (72.15%), *Supella longipalpa* (2.75%), *Periplaneta brunnea* (0.78%), *Periplaneta australasiae* (0.78%), *Neostylopyga rhombifolia* (0.78%) and *Blattella germanica* (0.39%) [22].

The National Cooperative Inner City Asthma Studies have established that cockroach allergy affects 37% of asthmatic children that are sensitized and exposed to cockroach allergens in the US [14;15]. Cockroach exposure and sensitivity predominate in the Northeast, with the highest values in Bronx, New York and Dallas of up to 81% and the highest cockroach allergen levels in high-rise apartments [23]. In the rest of the world, sensitization to cockroach allergens is high (38-80%) in South America (Brazil), Africa (Lagos) and Asian Countries (Malaysia, China, Singapore, Taiwan, Thailand) [22;24-31], whereas in Europe (France, Germany, Italy, Norway, Poland, Spain), Republic of Korea and Japan lower values have been reported (4-26%) [20;32-41].

## RELATIVE IMPORTANCE OF COCKROACH ALLERGENS

Since Bla g 2 was cloned in 1995, eight groups of cockroach allergens have been added to the WHO/IUIS list of Allergen Nomenclature, including the *B. germanica* allergens Bla g 1, Bla g 2, Bla g 4, Bla g 5, Bla g 6, Bla g 7 and Bla g 8, and the *P. americana* allergens Per a 1, Per a 2, Per a 3, Per a 6 and Per a 7, Table 1. Groups 1 and 7 are the only ones described as cross-reactive. Although experimental evidence indicates that most of the remaining allergens are species specific, the presence of low homologous variants cannot be discarded. For example, environmental studies show lack of allergen using a Bla g 2-specific ELISA in areas infested mostly with other cockroach species (*Periplaneta americana*, *Supella longipalpa*, *Periplaneta brunnea*, *Periplaneta australasiae* and *Neostylopyga rhombifolia*) in Thailand [22]. However, assays that measure Bla g 2 may not be able to recognize isoallergens with low degree of homology. In fact, recently, a Bla g 2 homolog from *P. americana* with only 44% identity has been identified by Dr. FT Chew [42].

The prevalence of IgE antibodies to the German cockroach allergens was measured in cockroach allergic patients with asthma living in the USA. Thirty and 57% of 106 sera had detectable IgE antibodies to natural Bla g 1 and Bla g 2, respectively. Among patients with low RAST (<200 RAST units/ml; n=48), the prevalence was lower (19 and 42%, respectively), reaching up to 40 and 70% among patients with high RAST (>200 RAST units/ml; n=58) [8]. In another study with 73 patients, recombinant Bla g 4 was recognized by 41% of the patients with high RAST, and 31% of patients with a RAST value of 40-200 units/ml [43]. The IgE binding to the natural and recombinant Bla g 5 was 68 and 73%, respectively (n=40) [44]. Interestingly, sensitization in 95% of *B. germanica* allergic patients was demonstrated by measuring IgE binding to Bla g 1, Bla g 2, Bla g 4 and Bla g 5 [44]. Biological reactivity of these allergens was proven by in-

tradermal skin testing, showing positive immediate skin reactions (>8 x 8 mm wheal) down to  $10^{-3}$ - $10^{-5}$  µg/ml of the purified natural allergens Bla g 1 and Bla g 2, rBla g 4 and rBla g 5. Several Per a 3 isoforms eliciting different skin reactivities (26-95%) were reported, with an 83% prevalence of IgE antibodies [45;46].

Recently, groups 6 and 8 of cockroach allergens have been described as homologs of troponin C and myosin light chain, respectively [47]. Three Bla g 6 isoallergens were cloned from a *Blattella germanica* cDNA expression library. The recombinant proteins expressed in *Pichia pastoris* showed a 14% prevalence of IgE antibodies. Interestingly, Bla g 6, Per a 6 and Bla g 8 are calcium binding proteins, and the IgE binding to Bla g 6 has proven to be calcium dependent [47].

Finally, the group 7 comprises homologs of tropomyosin, a pan-allergen involved in muscle contraction [48]. Bla g 7 and Per a 7 are included in this group, Table 1. The dusky brown cockroach (*Periplaneta fuliginosa*) also produces a tropomyosin with 100% and 98.2% amino acid identity to the *P. americana* and *B. germanica* homologs, respectively [50].

A recent study reported the relative importance of five cockroach allergens [51]. IgE antibodies to rBla g 1, rBla g 2, rBla g 4, rBla g 5 and rPer a 7 were measured in 118 sera from CR-sensitized subjects by streptavidin CAP assay. Unlike mite allergic patients, who are mostly sensitized to allergens from groups 1 and 2, the pattern of IgE responsiveness was unique to each subject, irrespective of geographic region within the U.S. The highest prevalence of IgE antibodies was to rBla g 2 (54%), which was approximately two fold higher than the prevalence of IgE antibodies to any of the other four allergens tested: 26% to rBla g 1, 17% to rBla g 4, 37% to rBla g 5 and 13% to rPer a 7 [51]. Among sera with high IgE levels to CR extract (3.5 to 100 IU/ml) the prevalence of IgE antibodies to rBla g 2 and rBla g 5 was 71% and 58%, respectively. These results show that Bla g 2 is the most important known German cockroach allergen, with positive intradermal skin tests at concentrations as low as  $10^{-3.5}$  µg/ml [8]. Bla g 2 is able to induce IgE production at levels of exposure 10-100 fold lower (<1 µg/g dust) than cat and mite allergens [13]. As mentioned above, Bla g 1 and Bla g 2 are used as markers of cockroach allergen exposure in environmental and avoidance studies. Exposure to low levels of Bla g 1 and Bla g 2 has been associated with wheezing among infants in the first 3 months of life and with increased proliferative T cell responses [52]. Resolution of Bla g 2 crystal structure revealed structural features that contribute to the allergenicity of this potent allergen, and will be described below in a specific section.

An arginine kinase from *P. americana* has recently been identified as a major cockroach allergen for cockroach allergic Thai patients, and has been assigned to group 9 by the WHO/IUIS Subcommittee of Allergen Nomenclature [53]. The protein has been cloned by Dr. FT Chew and the full sequence appears in the GenBank under accession number AY563004. All patients tested (n=25) reacted to the purified 40 kDa allergen by western blot. An arginine kinase homolog from *Blattella germanica* has also been reported in

Table 1. Systematic Nomenclature and Functional Properties of Cockroach Allergens

| <i>Blattella germanica</i>   |        |                           |                       |              |
|------------------------------|--------|---------------------------|-----------------------|--------------|
| Allergen                     | M.W.*  | Function/homology         | Accession number      | Reference    |
| Bla g 1                      | ~25-90 | Midgut microvilli         |                       |              |
| Bla g 1.0101                 | 46, 21 | protein homolog           | AF072219,<br>AF072221 | [58]         |
| Bla g 1.0102                 | 25-37  |                           | L47595                | [57]         |
| Bla g 1.02                   | 56     |                           | AF072220              | [58]         |
| Bla g 2**                    | 36     | Unusual aspartic protease | U28863                | [8;42;70]    |
| Bla g 4                      | 21     | Lipocalin                 | U40767                | [43]         |
| Bla g 5                      | 23     | Glutathione S-Transferase | U92412                | [44]         |
| Bla g 6                      | 17     | Troponin C                |                       |              |
| Bla g 6.0101                 |        |                           | DQ279092              | [47]         |
| Bla g 6.0201                 |        |                           | DQ279093              | [47]         |
| Bla g 6.0301                 |        |                           | DQ279094              | [47]         |
| Bla g 7                      | 33     | Tropomyosin               | AF260897              | [49]         |
| Bla g 8                      |        | Myosin light chain        | DQ389157              | [47]         |
| <i>Periplaneta americana</i> |        |                           |                       |              |
| Allergen                     | M.W.*  | Function/homology         | Accession number      | Reference    |
| Per a 1                      | ~25-45 | Midgut microvilli         |                       |              |
| Per a 1.0101                 | 26     | protein homolog           | AF072222              | [59]         |
| Per a 1.0102                 | 26     |                           | U78970                | [60]         |
| Per a 1.0103                 | 45     |                           | U69957                | [93]         |
| Per a 1.0104                 | 31     |                           | U69261                | [60]         |
| Per a 1.02                   | 51     |                           | U69260                | [60]         |
| Per a 2                      | 36     | Unusual aspartic protease | AY792947              | FT Chew [42] |
| Per a 3                      | 46-79  | Arylphorin/hemocyanin     |                       |              |
| Per a 3.01                   | 79     |                           | L40818                | [45]         |
| Per a 3.0201                 | 75     |                           | L40820                | [45]         |
| Per a 3.0202                 | 56     |                           | L40819                | [45]         |
| Per a 3.0203                 | 46     |                           | L40821                | [45]         |
| Per a 6                      | 17     | Troponin C                | AY792950              | FT Chew [47] |
| Per a 7                      | 33     | Tropomyosin               |                       |              |
| Per a 7.0101                 |        |                           | Y14854                | [89]         |
| Per a 7.0102                 |        |                           | AF106961              | [24]         |
| Per a 9                      |        | Arginine kinase           |                       |              |
| Per a 9.0101                 | 40     |                           | AY563004              | [53]         |

\* Molecular weight calculated from sequence.

\*\*The accession number for Bla g 2 in the Protein Data bank is 1YG9.

the GenBank (accession number ABC86902). Arginine kinase was first described as an allergen in black tiger shrimp (*Penaeus monodon*), showing 100% reactivity by intradermal skin test in six shrimp allergic patients using very small amounts of Pen m 2 (100µl of  $1 \times 10^{-9}$  or  $5 \times 10^{-9}$  M) [54]. This enzyme catalyzes the reversible transfer of the high energy phosphoryl group from ATP to arginine, yielding, apart from ADP, N-phosphoarginine which represents an intermediate storage and transport form of energy in a wide variety of invertebrates [54]. Der p 20 is an arginine kinase from dust mite reported in the WHO/IUIS list of Allergens, and the arginine kinase, Plo i 1, from the Indianmeal moth *Plodia interpunctella* has also been described. Inhibition experiments using dust mite, cockroach, king prawn, lobster and mussel extracts suggested that arginine kinase is an invertebrate panallergen [55].

Other proteins from *P. americana* have been cloned that share homology to the cockroach groups 2, 4 and arginine kinase (AY792947, AY792948 and AY563004) [42], and to the mite allergens from groups 2, 3 (trypsin) and 13 (fatty acid binding protein) (GenBank accession numbers AY792953, AY792954 and AY792955). However, the characterization of these proteins as allergens has not yet been reported. It will be interesting to know the prevalence of IgE antibodies in populations exposed to American cockroach. Finally, the literature reports the existence of other relatively high-molecular weight (MW) cockroach allergens that have not yet been cloned or identified [56;57]. Additional studies about the relative importance of different cockroach allergens in a wider range of populations are lacking, and would give a better insight on sensitization depending on allergen exposure, geographic location and genetic background.

In the following sections, we will describe what is known about the structure and function of different cockroach allergens, and its relationship to disease.

### 1. The Group 1 of Cross-Reactive Cockroach Allergens

Molecular cloning of Bla g 1 and Per a 1 revealed the existence of the Group 1 cockroach allergens [57-60]. Bla g 1 and Per a 1 were the first cross-reactive cockroach allergens to be identified, sharing a 70-72% amino acid sequence identity. Together with Bla g 2, they have been used as markers for cockroach allergen exposure in environmental studies.

The novel feature of the Bla g 1 and Per a 1 primary structure is that they contain multiple tandem repeats of ~100 amino acid residues. The sequences described form a family of structurally and antigenically related proteins, which differ in the number of amino acid repeats they contain. Dot-plot sequence analysis revealed that two consecutive amino acid repeats from Bla g 1 are encoded by one nucleotide repeat. The homology between alternative repeats (first and third or second and fourth) is higher than between consecutive repeats, both at the nucleotide and amino acid level, Fig. (1). This phenomenon indicates that an amino acid duplex would have originated by gene duplication of an original DNA encoding for a 100 amino acid domain. Both amino acid repeats in a duplex would be recognized by dot plot analysis, but the corresponding nucleotide sequence would only represent one repeat due to the divergence in the nu-

cleotide sequence [58]. N-terminal sequencing of the natural allergen suggested that it is cleaved by trypsin-like enzymes after arginine residues, resulting in a mixture of molecules of different size containing a different number of amino acid repeats, which are recognized by IgE [61].

#### Homology of Bla g 1.0101 repeats

|                       |         | % Amino acid identity |        |       |        |
|-----------------------|---------|-----------------------|--------|-------|--------|
|                       |         | First                 | Second | Third | Fourth |
| % Nucleotide identity | REPEATS | First                 | Second | Third | Fourth |
|                       | First   | 100                   | 26     | 98    | 28     |
|                       | Second  | 45                    | 100    | 27    | 96     |
|                       | Third   | 95                    | 44     | 100   | 29     |
|                       | Fourth  | 50                    | 91     | 50    | 100    |

**Figure 1.** Sequence homology, as percentage of identity, among repeats of the cockroach allergen Bla g 1.0101, at the nucleotide and amino acid level (left and right from the diagonal, respectively).

The Group 1 cockroach allergens show homology to microvillar membrane associated proteins from the coleopteran *Tenebrio molitor* (GenBank accession number AY327800) and the mosquito *Aedes aegypti* (AEG12) (AY038041, AY050565) [62;63]. They also share homology with a protein from *Anopheles gambiae*, ANG12, which, like AEG12, is a protein induced after blood meal in the midgut of adult female mosquitoes (accession number Z22925). Similarly, Bla g 1 could have a still unknown digestive function, since it is secreted in the digestive tract of the cockroach and is most prevalent in the midgut, being the Bla g 1 gene exclusively expressed in midgut cells [58;64]. Adult females produce and excrete significantly more Bla g 1 in their feces than males and nymphs, most likely because females process more food and production is related to food intake [65].

### 2. The Atypical Aspartic Protease Bla g 2

Bla g 2 is a very potent allergen eliciting IgE antibody responses in ~60% of cockroach allergic patients at low levels of exposure [8;13]. Bla g 2 is a 36 kDa protein that shares homology (~28% amino acid identity) with the aspartic protease group of enzymes (renin, pepsin, chymosin...). As Bla g 1, Bla g 2 was shown to be present in highest concentration in the digestive system, principally esophagus, proventriculus and gut [8]. The crystal structure of Bla g 2 was recently solved and shows a bilobal shape, typical of aspartic prote-

ases, with two domains of different amino acid sequence, but basically similar folds, and a cleft between them that contains the catalytic site, Fig. (2). There is evidence that this bilobal shape was evolutionarily derived from gene duplication [66]. In fact, the viral aspartic proteases (e.g. from HIV) still preserve the original dimeric structure. The carbon alpha coordinates of the Bla g 2 structure deviate only  $\sim 1.5$  Å from those of active enzymes, such as pepsin and chymosin, indicating that Bla g 2 has a very similar folding to the active enzymes. A way to compare protein folds and domain interactions, without rotating the molecules, is by constructing proximity plots of the molecules [67]. The similar folding of Bla g 2 and homologous aspartic proteases is reflected in equivalent proximity plots, Fig. (3). The bottom left and top right part of the plot reflects the two lobes characteristic of aspartic proteases.

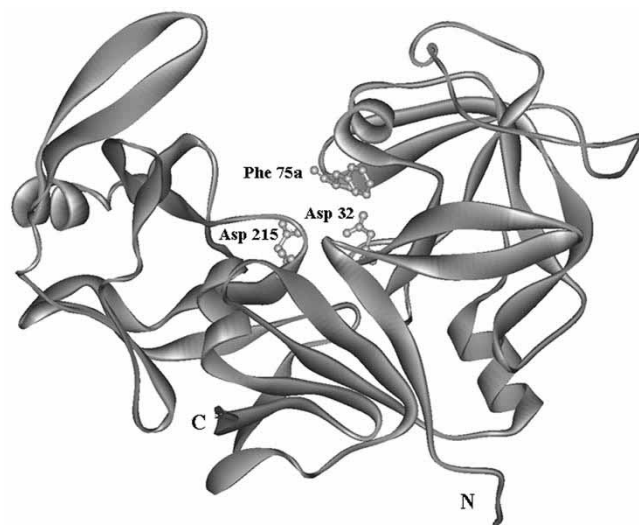


**Figure 2.** Ribbon representation of the crystal structure of Bla g 2 (1YG9.pdb), showing the catalytic aspartates 32 and 215 at the bottom of the cleft, and the insertion of phenylalanine 75a in the “flap” region.

The catalytic site of aspartic proteases is formed by two loops, each containing the motif DS/TG, at the bottom of the cleft or binding pocket. The catalytic aspartates in the motif, far apart in the primary sequence (32 and 215 following pepsin numbering), are very close in the folded molecule, and are essential for catalysis interacting with the substrate through a water molecule, Fig. (2). Interestingly, Bla g 2 is unusual due to important amino acid substitutions of the glycines by serine or threonine, so the first and second triads are DST and DTS, respectively [68]. In addition, there is an insertion (F75a) in the “flap” that interferes with the typical substrate binding site, providing an autoinhibitory mechanism of enzymatic activity, Fig. (2). The “flap” is a  $\beta$ -hairpin (residues 72–80) that partially covers the active site cleft, acting like a “lid” and contributing to the specificity of the enzyme for substrates. F75a and Asp76 are hydrogen bonded to the catalytic aspartates 32 and 215, respectively. These interactions place the “flap” into a completely “closed” conformation and make the catalytic aspartates less accessible. No direct contacts between these aspartates and the solvent molecules have been observed in Bla g 2 crystals.

Bla g 2 strikingly resembles a group of inactive aspartic proteases called pregnancy associated glycoproteins (PAG), secreted in the chorion of female ruminants during pregnancy [69]. PAG have similar amino acid substitutions to Bla g 2 in the catalytic area [69]. For example, alanine rather than glycine is present in the first catalytic motif of boPAG-1 and poPAG-1, and glycine instead of aspartate is found in the second motif of ovPAG-1. An extra phenylalanine is also found in ovPAG-1. Despite the homology to aspartic proteases, Bla g 2 and PAG have important substitutions in the area corresponding to the active site that are expected to impair enzymatic activity [68].

The crystal structure of Bla g 2 also revealed that distortions in the catalytic site due to these substitutions lead to a bigger distance of the catalytic aspartates (4.41 Å instead of the usual  $\sim 3$  Å), which is expected to impair enzymatic activity [42]. Accordingly, standard aspartic protease assays based on the proteolytic cleavage of casein from milk or hemoglobin as substrates showed undetectable enzymatic activity in Bla g 2 [70]. In addition, pepstatin, a very common aspartic protease inhibitor, was unable to specifically bind Bla g 2, proving the atypical conformation of the Bla g 2 binding pocket [68].

Two more unique structural characteristics may contribute to Bla g 2 allergenicity by conferring stability to the molecule: a) the allergen binds one atom of zinc through residues His155, His161, Asp303 and Asp307, and b) Bla g 2 has five di-sulfide bonds in contrast to only two or three in active aspartic proteases.

The identity of the metal bound in the crystal of Bla g 2 was established by x-ray absorption spectroscopy (EXAFS) near the absorption edges of Ni and Zn, performed on small crystals of the protein mounted in a capillary. Only one of the aspartates (Asp303) is conserved in other enzymes, while the three remaining residues are unique to Bla g 2, explaining why zinc has not previously been seen in the structures of aspartic proteases. Zinc binding may be a cockroach specific feature, since the four residues have also been observed in the amino acid sequence of the *Periplaneta americana* and *Leucophaea maderae* Bla g 2-homologs. Zinc binding may contribute to the stability of Bla g 2 by keeping together two secondary structure elements (a  $\beta$ -loop and an  $\alpha$ -helix) that contain the histidines and aspartates, respectively.

Bla g 2 has five di-sulfide bonds, in contrast to only two or three in active aspartic proteases. The bonds are between the cysteines: 36–127, 45–50 and 51a–113 in the N-terminal domain, and 237–245 and 249–282, in the C-terminal domain. Only two of them, 45–50 and 249–282, are conserved within the whole pepsin family. The remaining three disulfide bonds, 36–127, 51a–113, and 237–245, do not have structural analogs in the other aspartic proteases described to date. A disulfide bond located between 206–210 in pepsin, chymosin, renin and most other aspartic proteases does not have an equivalent in Bla g 2 (or in proteinase A).

In summary, the presence of a zinc binding site in Bla g 2 that provides additional interactions between two neighboring secondary structure elements, as well as the existence of five disulfide bridges, increases the stability of the allergen structure and may influence its persistence in the environ-

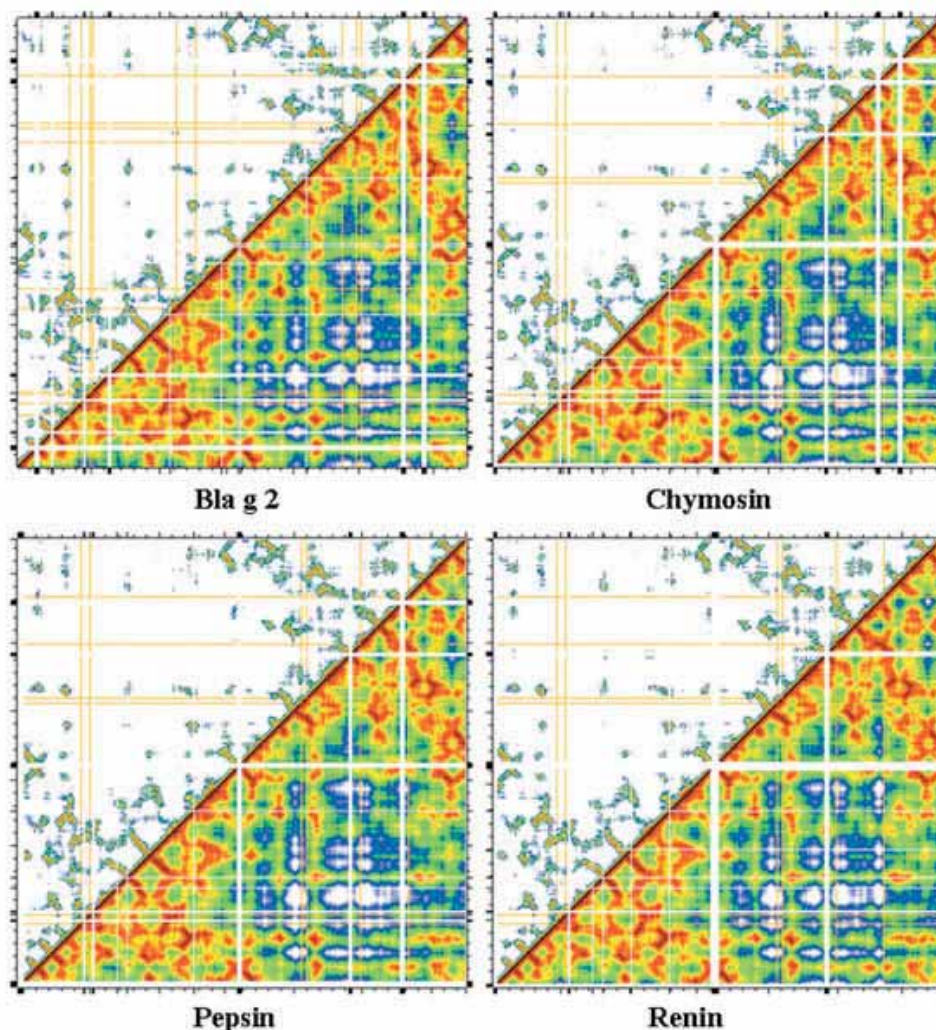


**Figure 3.** Proximity plots of Bla g 2 and the active aspartic protease homologs chymosin, pepsin and renin based on a structural alignment of the amino acid sequences [42]. These plots show the distances among the alpha-carbons of a protein, which are zero for the diagonal [67]. The white lines in the plots indicate amino acid gaps in the protein sequence.

ment. Chronic exposure to low doses (1-10 µg/year) of this stable cockroach allergen may explain why sensitization and exposure to Bla g 2 is associated with asthma [13;71].

### 3. Per a 3, A Hemolymph Homolog

Per a 3 has only been described in *P. americana* as a group of allergens that share homology with arylphorins or insect storage proteins (20-34%), insect juvenile hormone-suppressible proteins (31-36%) and arthropod hemocyanins (30-35%) [45]. Two high molecular weight isoallergens (Per a 3.01 and Per a 3.0201, with 78 and 72 kDa, respectively) showed 82.6% prevalence of IgE antibodies, with 47.4% skin reactivity in 19 cockroach-sensitive asthmatic patients. Two more variants (Per a 3.0202 and Per a 3.0203, with 56.2 and 46.7 kDa, respectively) have been identified with 26 and 95% skin reactivity, respectively, confirming the importance of this allergen in *P. americana*, Table 1 [45,46].

### 4. The Insect Lipocalin Bla g 4

The molecular cloning of Bla g 4 revealed the existence of an insect homolog to a family of proteins, the lipocalins,

that includes important mammalian allergens: Bos d 2 and Bos d 5 from cow; Can f 1 and Can f 2 from dog; Equ c 1 and Equ c 2 from horse; Fel d 4 from cat and Mus m 1 and Rat n 1 from mouse and rat, Fig. (4) [72-77]. Except for Bos d 5 which is  $\beta$ -lactoglobulin from milk, most lipocalin allergens are inhaled. Lipocalins are small extra-cellular proteins present in secretions such as saliva or urine. Despite an amino acid identity as low as 20%, the crystal structures of lipocalins show the same characteristic lipocalin folding: a C-terminal  $\alpha$ -helix and a single eight-stranded (ten-stranded for Bos d 5) antiparallel  $\beta$ -barrel enclosing an internal cavity, Fig. (4). The function of lipocalins is related to their capacity to bind small hydrophobic ligands such as pheromones, steroids, retinoids and arachidonic acid [78]. Bos d 5 binds palmitate and retinol, and the rodent urinary proteins Mus m1 and Rat n 1 bind pheromones [73;76;79;80].

Recent studies establish that Bla g 4, an 18 kDa lipocalin or transport binding protein, has a male reproductive function. Bla g 4 is only expressed in the adult male reproductive system, specifically in the utricles and the conglobate gland, and is transferred to the female within the spermatophore

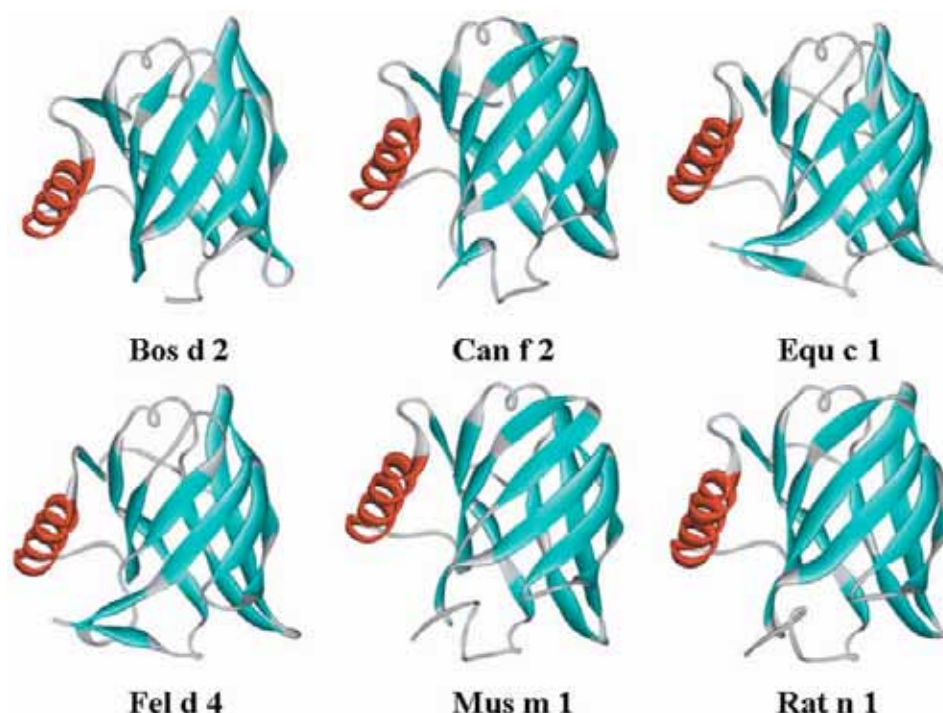


**Figure 4.** Tertiary structure of mammalian lipocalin allergens based on the crystal structures from cow (Bos d 2; from the Protein Data Bank file 1BJ7.pdb), horse (Equ c 1; 1EW3.pdb), mouse (Mus m 1; 1MUP.pdb) and rat (Rat n 1; 2A2U.pdb), and molecular models of Can f 2 and Fel d 4 [75;92].

during copulation [81]. Bla g 4 would be secreted with other seminal secretions during sexual activity. The presence of Bla g 4 in the discarded spermatophore expelled by the mated female suggests that Bla g 4 may be a component of the spermatophore rather than the seminal secretions. These studies demonstrate that Bla g 4, together with Bla g 1 and Bla g 2, are allergens secreted or excreted by cockroaches. These dried secretions could be the form in which cockroach allergens become airborne and cause sensitization in genetically predisposed individuals. The accumulation and persistence of stable cockroach allergens in the home could result in low levels of chronic exposure that is associated with asthma. It is also possible that the biological function of the allergen could have direct effects on the lung.

### 5. Bla g 5: A Glutathione Transferase Homolog

Bla g 5 is a 23 kDa allergen that was assigned to the glutathione S-transferase superfamily (GST) of proteins based on sequence homology (42-51% identity) to the GST-2 subfamily from insects, including *Anopheles gambiae* and *Drosophila melanogaster* [44]. Glutathione S-transferases are found in most forms of life and are involved in the detoxification of endogenous and xenobiotic toxic compounds. Resistance to insecticides is associated with up-regulation of GST production in insects. Therefore, attempts to exterminate cockroaches in homes of allergic patients could lead to an undesired increase of the expression and environmental exposure to Bla g 5 [44].

Low enzymatic activity was detected in the recombinant allergen expressed in *E. coli* and using the GST substrate CDNB (1-chloro-2,4-dinitrobenzene). The capacity of Bla g

5 to bind glutathione has been used for purification of this allergen.

Other allergens that are glutathione S-transferase homologs are Der p 8 from dust mite and Alt a 13 from *Alternaria alternata*. Antigenic cross-reactivity has been reported between mite and cockroach GST [82].

### 6. Cockroach Allergens with Homology to Proteins Involved in Contraction: Groups 6, 7 and 8

Tropomyosin was first identified as an allergen in shrimp [83-85]. Due to the ubiquitous presence of this protein in animal muscle, other cross-reactive tropomyosin allergens were described afterwards in arthropods (mollusks, arachnids, insects), and is now considered a pan-allergen [48]. The WHO-IUIS list of Allergen Nomenclature includes the following tropomyosin allergens: cockroach (Group 7), mite (Group 10), shrimp (Met e 1, Pen a 1, Pen i 1), squid (Tod p 1), crab (Cha f 1), midge (Chi k 10), nematode (Ani s 3), lobster (Hom a 1, Pan s 1) and snail (Hel a 1). The molecular structure of tropomyosin consists of two parallel  $\alpha$ -helical molecules wound around each other. The high degree of homology (~80%) is the basis for the antigenic cross-reactivity observed among these allergens [86;87]. However, these allergens from invertebrate origin show lower homology with vertebrate tropomyosins (~45% identity). The lack of cross-reactivity between vertebrate and invertebrate tropomyosins would be explained by the presence of IgE binding epitopes in parts of the allergen non-homologous to the vertebrate tropomyosins [83,88].

The group 7 of cockroach allergens comprises Bla g 7 and Per a 7. As mentioned above, the dusky brown cock-

roach (*Periplaneta fuliginosa*) also produces a tropomyosin highly homologous to the German and American cockroach tropomyosins [50]. The prevalence of sensitization to rBla g 7 and rPer a 7 was 16% by ELISA and 50% by plaque immunoassay, respectively [24;49;89]. Plaque immunoassay tends to overestimate values, and a recent study that compared IgE reactivity to five cockroach allergens by streptavidin CAP assay in 118 sera measured a 13% prevalence of IgE antibodies to rPer a 7 [51]. Only 16% of cockroach-sensitized subjects with IgE antibodies to house dust mite exhibited IgE antibody binding to rPer a 7. This result suggests that concomitant exposure to house dust mite and cockroach allergens other than tropomyosin, instead of antigenic cross-reactivity to tropomyosin, explains most of the co-sensitization to mite and cockroach [51].

Recently, new cockroach allergens have been cloned and assigned to groups 6 and 8. These allergens show homology to troponin C and myosin light chain, respectively [47]. As mentioned above, Bla g 6 comprises three isoallergens with a 14% prevalence of IgE antibodies. Troponin C is part of a complex with two more subunits: troponin I (the inhibitory subunit) and troponin T (the tropomyosin-binding subunit). The troponin complex, together with tropomyosin, is located on the actin filament. The molecular structure of troponin C consists on two globular lobes of  $\alpha$ -helices connected by a flexible linker. The C-lobe integrates the protein into the troponin complex, whereas the N-terminal lobe has a regulatory function for which calcium binding to the EF-hand calcium motifs is essential. These motifs consist of a 12 amino acid binding loop flanked by two perpendicular  $\alpha$ -helices [90]. While troponin C binds three and four  $\text{Ca}^{2+}$  in vertebrate cardiac and skeletal muscle, respectively, troponin C from arthropods (including cockroaches) and mollusks binds only two  $\text{Ca}^{2+}$ , one in each of the two terminal lobes present in the molecule. In presence of calcium the linker is a long  $\alpha$ -helix, and troponin I is bound to troponin C. When calcium is low, the  $\alpha$ -helix bends 90°, releases troponin I and this unit inhibits actomyosin ATPase by interacting with actin. Interestingly, the IgE binding to Bla g 6 has proven to be calcium dependent, with highest values when calcium was added to calcium-depleted allergen [47]. This result indicates that IgE production was preferably induced against one of the molecular conformations.

Finally, Bla g 8 shares 81-84% amino acid sequence identity with the myosin light chains of the caterpillar *Lononia obliqua*, the cricket *Gryllotalpa orientalis* and the silkworm *Bombyx mori*, and 60-66% identity to myosin light chains of fly (*Drosophila*) and mosquito (*Anopheles* and *Aedes*) species. The myosin regulatory light chains are small acidic polypeptides non-covalently bound to the neck region of the myosin head, which regulate the interaction of the myosin head with actin [91]. Their regulatory function is also mediated by  $\text{Ca}^{2+}$  binding to one EF-hand motif.

## FINAL NOTE

Cockroach allergens comprise a wide group of proteins with diverse structure and biological function. They are released to the environment either from the digestive tract by excretion with feces (groups 1 and 2), or secreted from the adult male reproductive system during sexual activity (Bla g

4), or from dead bodies by degradation of tissues such as muscle (groups 6, 7 and 8). Carried by particles of 10-40  $\mu\text{m}$  in diameter, they reach the lung and are able to induce IgE responses in genetically predisposed individuals. Cockroach allergens, classified in nine groups, show homology to microvillar proteins, aspartic proteases, arylphorin/hemocyanins, lipocalins, glutathione S-transferases, troponin C, tropomyosins, myosin light chain and arginine kinase. Resolution of the tertiary structure of these allergens will allow a rational design of hypoallergenic forms for immunotherapy. The determination of antigenic determinants on the allergen surface will be crucial to understand phenomena of cross-reactivity, as well as the IgE and IgG antibody responses to cockroach allergens in different populations. Unlike other allergens (mite, cat, pollens) no efforts for standardization of cockroach allergens have been made, and expression of recombinant cockroach allergens will facilitate this process. In summary, knowledge of the intrinsic properties of cockroach allergens will provide new insights on allergenicity and the basis to design tools for diagnosis and immunotherapy of allergic disease.

## ACKNOWLEDGEMENTS

We thank Dr. R. Aalberse for providing the means to construct the aspartic protease proximity plots. This work is supported by INDOOR Biotechnologies, Inc.

## LIST OF ABBREVIATIONS

- GST = Glutathione S-transferase  
 PAG = Pregnancy associated glycoproteins  
 RAST = Radio immunoallergosorbent test

## REFERENCES

- [1] Bernton, H.S. and Brown, H. (1964) *J. Allergy Clin. Immunol.*, 35, 506-513.
- [2] Bernton, H.S., McMahon, T.F. and Brown, H. (1972) *Br. J. Dis. Chest*, 66, 61-66.
- [3] Kang, B., Vellody, D., Homburger, H. and Yunginger, J.W. (1979) *J. Allergy Clin. Immunol.*, 63, 80-86.
- [4] Chapman MD, Vailes, L.D., Hayden, M., Platts-Mills, T. and Arruda, L. (1997) in *Allergy and Allergic Diseases* (Kay, A.B., Ed.), pp., 942-951., Blackwell Science Ltd., Oxford.
- [5] Platts-Mills, T.A., Vervloet, D., Thomas, W.R., Aalberse, R.C. and Chapman, M.D. (1997) *J. Allergy Clin. Immunol.*, 100, S2-24.
- [6] Christiansen, S.C., Martin, S.B., Schleicher, N.C., Koziol, J.A., Hamilton, R.G. and Zuraw, B.L. (1996) *J. Allergy Clin. Immunol.*, 98, 288-294.
- [7] Pollart, S.M., Chapman, M.D., Fiocco, G.P., Rose, G. and Platts-Mills, T.A. (1989) *J. Allergy Clin. Immunol.*, 83, 875-882.
- [8] Arruda, L.K., Vailes, L.D., Mann, B.J., Shannon, J., Fox, J.W., Vedvick, T.S., Hayden, M.L. and Chapman, M.D. (1995) *J. Biol. Chem.*, 270, 19563-19568.
- [9] Huss, K., Adkinson, N.F. Jr., Eggleston, P.A., Dawson, C., Van Natta, M.L. and Hamilton, R.G. (2001) *J. Allergy Clin. Immunol.*, 107, 48-54.
- [10] Matsui, E.C., Wood, R.A., Rand, C., Kanchanaraks, S., Swartz, L., Curtin-Brosnan, J. and Eggleston, P.A. (2003) *J. Allergy Clin. Immunol.*, 112, 87-92.
- [11] Call, R.S., Smith, T.F., Morris, E., Chapman, M.D. and Platts-Mills, T.A. (1992) *J. Pediatr.*, 121, 862-866.
- [12] Lehrer, S.B., Horner, W.E., Menon, P. and Stankus, R.P. (1991) *J. Allergy Clin. Immunol.*, 87, 574-580.
- [13] Sporik, R., Squillace, S.P., Ingram, J.M., Rakes, G., Honsinger, R.W. and Platts-Mills, T.A. (1999) *Thorax*, 54, 675-680.

- [14] Rosenstreich, D.L., Eggleston, P., Kattan, M., Baker, D., Slavin, R.G., Gergen, P., Mitchell, H., Niff-Mortimer, K., Lynn, H., Ownby, D. and Malveaux, F. (1997) *N. Engl. J. Med.*, 336, 1356-1363.
- [15] Eggleston, P.A., Rosenstreich, D., Lynn, H., Gergen, P., Baker, D., Kattan, M., Mortimer, K.M., Mitchell, H., Ownby, D., Slavin, R. and Malveaux, F. (1998) *J. Allergy Clin. Immunol.*, 102, 563-570.
- [16] Eggleston, P.A., Wood, R.A., Rand, C., Nixon, W.J., Chen, P.H. and Lukk, P. (1999) *J. Allergy Clin. Immunol.*, 104, 842-846.
- [17] Arbes, S.J., Jr., Sever, M., Mehta, J., Gore, J.C., Schal, C., Vaughn, B., Mitchell, H. and Zeldin, D.C. (2004) *J. Allergy Clin. Immunol.*, 113, 109-114.
- [18] Grandcolas, Ph. (1998) *Rev. Fr. Allergol.*, 38, 833-838.
- [19] Appel, A.G. and Smith, L.M. (2002) *Annu. Rev. Entomol.*, 47, 33-55.
- [20] Sakaguchi, M., Inouye, S., Miyazawa, H., Okabe, T., Yasueda, H., Muto, A., Tanaka, I., Akasaka, T., Wu, C.H., Chapman, M.D. (1994) *Arerugi*, 43, 1309-1315.
- [21] Lee, D.K., Lee, W.J. and Sim, J.K. (2003) *J. Vector Ecol.*, 28, 90-96.
- [22] Tungtrongchitr, A., Sookrung, N., Munkong, N., Mahakittikun, V., Chinabut, P., Chaicumpa, W., Bunnag, C. and Vichyanond, P. (2004) *Asian Pac. J. Allergy Immunol.*, 22, 115-121.
- [23] Gruchalla, R.S., Pongracic, J., Plaut, M., Evans, R., III Visness, C.M., Walter, M., Crain, E.F., Kattan, M., Morgan, W.J., Steinbach, S., Stout, J., Malindzak, G., Smartt, E. and Mitchell, H. (2005) *J. Allergy Clin. Immunol.*, 115, 478-485.
- [24] Santos, A.B., Chapman, M.D., Aalberse, R.C., Vailes, L.D., Ferriani, V.P., Oliver, C., Rizzo, M.C., Naspitz, C.K. and Arruda, L.K. (1999) *J. Allergy Clin. Immunol.*, 104, 329-337.
- [25] Adanijo, A.O. and Bandele, E.O. (2000) *East Afr. Med. J.*, 77, 622-626.
- [26] Sam, C.K., Soon, S.C., Liam, C.K., Padmaja, K. and Cheng, H.M. (1998) *Asian Pac. J. Allergy Immunol.*, 16, 17-20.
- [27] Chiang, C.H., Wu, K.M., Wu, C.P., Yan, H.C. and Perng, W.C. (2005) *J. Chin. Med. Assoc.*, 68, 204-209.
- [28] Lan, J.L., Lee, D.T., Wu, C.H., Chang, C.P. and Yeh, C.L. (1988) *J. Allergy Clin. Immunol.*, 82, 736-740.
- [29] Kongpanichkul, A., Vichyanond, P. and Tuchinda, M. (1997) *J. Med. Assoc. Thai*, 80, 69-75.
- [30] Pumhirun, P., Towiwat, P. and Mahakit, P. (1997) *Asian Pac. J. Allergy Immunol.*, 15, 183-185.
- [31] Chew, F.T., Lim, S.H., Goh, D.Y. and Lee, B.W. (1999) *Allergy*, 54, 1150-1159.
- [32] Birnbaum, J., Guilloux, L., Charpin, D. and Vervloet, D. (1991) *Lancet*, 337, 249.
- [33] Hirsch, T., Stappenbeck, C., Neumeister, V., Weiland, S.K., von, M.E., Keil, U. and Leupold, W. (2000) *Clin. Exp. Allergy*, 30, 529-537.
- [34] Peruzzi, M., de, L.M., Novembre, E., de, M.M. and Vierucci, A. (1999) *Ann. Allergy Asthma Immunol.*, 83, 167-171.
- [35] Lodrup Carlsen, K.C., Carlsen, K.H., Buchmann, M.S., Wikstrom, J. and Mehl, R. (2002) *Allergy*, 57, 529-533.
- [36] Stelmach, I., Jerzynska, J., Stelmach, W., Majak, P., Chew, G., Gorski, P. and Kuna, P. (2002) *Allergy*, 57, 701-705.
- [37] Sastre, J., Ibanez, M.D., Lombardero, M., Laso, M.T. and Lehrer, S. (1996) *Allergy*, 51, 582-586.
- [38] Hwang, K.Y., Park, J.S., Ahn, H.C. and Nam, H.S. (2001) *Korean. J. Parasitol.*, 39, 197-199.
- [39] Lee, M.H., Kim, Y.K., Min, K.U., Lee, B.J., Bahn, J.W., Son, J.W., Cho, S.H., Park, H.S., Koh, Y.Y. and Kim, Y.Y. (2001) *Ann. Allergy Asthma Immunol.*, 86, 691-695.
- [40] Okuda, M., Usami, A., Itoh, H. and Ogino, S. (2002) *Nippon Jibiinkoka Gakkai Kaiho*, 105, 1181-1188.
- [41] Tomita, S., Suzuki, H. and Akiyama, K. (2002) *Arerugi*, 51, 430-438.
- [42] Gustchina, A., Li, M., Wünschmann, S., Chapman, M.D., Pomés, A. and Wlodawer, A. (2005) *J. Mol. Biol.*, 348, 433-444.
- [43] Arruda, L.K., Vailes, L.D., Hayden, M.L., Benjamin, D.C. and Chapman, M.D. (1995) *J. Biol. Chem.*, 270, 31196-31201.
- [44] Arruda, L.K., Vailes, L.D., Platts-Mills, T.A., Hayden, M.L. and Chapman, M.D. (1997) *J. Biol. Chem.*, 272, 20907-20912.
- [45] Wu, C.H., Lee, M.F., Liao, S.C. and Luo, S.F. (1996) *J. Biol. Chem.*, 271, 17937-17943.
- [46] Wu, C.H., Lee, M.F., Wang, N.M. and Luo, S.F. (1997) *Mol. Immunol.*, 34, 1-8.
- [47] Hindley J Wünschmann S Satinover SM Woodfolk JA Chew FT Chapman MD and Pomés A (2006) *J. Allergy Clin. Immunol.*,
- [48] Reese, G., Ayuso, R. and Lehrer, S.B. (1999) *Int. Arch. Allergy Immunol.*, 119, 247-258.
- [49] Jeong, K.Y., Lee, J., Lee, I.Y., Ree, H.I., Hong, C.S. and Yong, T.S. (2003) *Allergy*, 58, 1059-1063.
- [50] Jeong, K.Y., Hwang, H., Lee, J., Lee, I.Y., Kim, D.S., Hong, C.S., Ree, H.I. and Yong, T.S. (2004) *Clin. Diagn. Lab. Immunol.*, 11, 680-685.
- [51] Satinover, S.M., Reefer, A.J., Pomés, A., Chapman, M.D., Platts-Mills, T.A. and Woodfolk, J.A. (2005) *J. Allergy Clin. Immunol.*, 115, 803-809.
- [52] Finn, P.W., Boudreau, J.O., He, H., Wang, Y., Chapman, M.D., Vincent, C., Burge, H.A., Weiss, S.T., Perkins, D.L. and Gold, D.R. (2000) *J. Allergy Clin. Immunol.*, 105, 933-942.
- [53] Sookrung, N., Chaicumpa, W., Tungtrongchitr, A., Vichyanond, P., Bunnag, C., Ramasoota, P., Tongtawe, P., Sakolvaree, Y. and Tapchaisri, P. (2006) *Environ. Health Perspect.*, 114, 875-880.
- [54] Yu, C.J., Lin, Y.F., Chiang, B.L. and Chow, L.P. (2003) *J. Immunol.*, 170, 445-453.
- [55] Binder, M., Mahler, V., Hayek, B., Sperr, W.R., Scholler, M., Prozell, S., Wiedermann, G., Valent, P., Valenta, R. and Duchene, M. (2001) *J. Immunol.*, 167, 5470-5477.
- [56] Musmand, J.J., Horner, W.E., Lopez, M. and Lehrer, S.B. (1995) *J. Allergy Clin. Immunol.*, 95, 877-885.
- [57] Helm, R., Cockrell, G., Stanley, J.S., Brenner, R.J., Burks, W. and Bannon, G.A. (1996) *J. Allergy Clin. Immunol.*, 98, 172-180.
- [58] Pomés, A., Melén, E., Vailes, L.D., Retief, J.D., Arruda, L.K. and Chapman, M.D. (1998) *J. Biol. Chem.*, 273, 30801-30807.
- [59] Melén, E., Pomés, A., Vailes, L.D., Arruda, L.K. and Chapman, M.D. (1999) *J. Allergy Clin. Immunol.*, 103, 859-864.
- [60] Wu, C.H., Wang, N.M., Lee, M.F., Kao, C.Y. and Luo, S.F. (1998) *J. Allergy Clin. Immunol.*, 101, 832-840.
- [61] Pomés, A., Vailes, L.D., Helm, R.M. and Chapman, M.D. (2002) *Eur. J. Biochem.*, 269, 3086-3092.
- [62] Morlais, I., Mori, A., Schneider, J.R. and Severson, D.W. (2003) *Mol. Genet. Genomics*, 269, 753-764.
- [63] Shao, L., Devenport, M., Fujioka, H., Ghosh, A. and Jacobs-Lorena, M. (2005) *Insect. Biochem. Mol. Biol.*, 35, 947-959.
- [64] Gore, J.C. and Schal, C. (2004) *J. Med. Entomol.*, 41, 953-960.
- [65] Gore, J.C. and Schal, C. (2005) *Med. Vet. Entomol.*, 19, 127-134.
- [66] Tang, J., James, M.N., Hsu, I.N., Jenkins, J.A. and Blundell, T.L. (1978) *Nature*, 271, 618-621.
- [67] Aalberse, R.C. (2000) *J. Allergy Clin. Immunol.*, 106, 228-238.
- [68] Pomés, A., Chapman, M.D., Vailes, L.D., Blundell, T.L. and Dhanaraj, V. (2002) *Am. J. Respir. Crit. Care Med.*, 165, 391-397.
- [69] Guruprasad, K., Blundell, T.L., Xie, S., Green, J., Szafranska, B., Nagel, R.J., McDowell, K., Baker, C.B. and Roberts, R.M. (1996) *Protein Eng.*, 9, 849-856.
- [70] Wünschmann, S., Gustchina, A., Chapman, M.D. and Pomés, A. (2005) *J. Allergy Clin. Immunol.*, 116, 140-145.
- [71] Arruda, L.K., Vailes, L.D., Ferriani, V.P., Santos, A.B., Pomés, A. and Chapman, M.D. (2001) *J. Allergy Clin. Immunol.*, 107, 419-428.
- [72] Rouvinen, J., Rautiainen, J., Virtanen, T., Zeiler, T., Kauppinen, J., Taivainen, A. and Mantylarvi, R. (1999) *J. Biol. Chem.*, 274, 2337-2343.
- [73] Wu, S.Y., Perez, M.D., Puyol, P. and Sawyer, L. (1999) *J. Biol. Chem.*, 274, 170-174.
- [74] Konieczny, A., Morgenstern, J.P., Bizinkauskas, C.B., Lilley, C.H., Brauer, A.W., Bond, J.F., Aalberse, R.C., Wallner, B.P. and Kasaian, M.T. (1997) *Immunology*, 92, 577-586.
- [75] Smith, W., Butler, A.J., Hazell, L.A., Chapman, M.D., Pomés, A., Nickels, D.G. and Thomas, W.R. (2004) *Clin. Exp. Allergy*, 34, 1732-1738.
- [76] Bocskei, Z., Groom, C.R., Flower, D.R., Wright, C.E., Phillips, S.E., Cavaggioni, A., Findlay, J.B. and North, A.C. (1992) *Nature*, 360, 186-188.
- [77] Chaudhuri, B.N., Kleywegt, G.J., Bjorkman, J., Lehman-McKeeman, L.D., Oliver, J.D. and Jones, T.A. (1999) *Acta Crystallogr. D. Biol. Crystallogr.*, 55, 753-762.
- [78] Flower, D.R., North, A.C. and Sansom, C.E. (2000) *Biochim. Biophys. Acta*, 1482, 9-24.
- [79] Futterman, S. and Heller, J. (1972) *J. Biol. Chem.*, 247, 5168-5172.
- [80] Lange, D.C., Kothari, R., Patel, R.C. and Patel, S.C. (1998) *Biophys. Chem.*, 74, 45-51.

- [81] Fan, Y., Gore, J.C., Redding, K.O., Vailes, L.D., Chapman, M.D. and Schal, C. (2005) *Insect. Mol. Biol.*, 14, 45-53.
- [82] Huang, C.H., Liew, L.M., Mah, K.W., Kuo, I.C., Lee, B.W. and Chua, K.Y. (2006) *Clin. Exp. Allergy*, 36, 369-376.
- [83] Shanti, K.N., Martin, B.M., Nagpal, S., Metcalfe, D.D. and Rao, P.V. (1993) *J. Immunol.*, 151, 5354-5363.
- [84] Daul, C.B., Slattery, M., Reese, G. and Lehrer, S.B. (1994) *Int. Arch. Allergy Immunol.*, 105, 49-55.
- [85] Leung, P.S., Chu, K.H., Chow, W.K., Ansari, A., Bandea, C.I., Kwan, H.S., Nagy, S.M. and Gershwin, M.E. (1994) *J. Allergy Clin. Immunol.*, 94, 882-890.
- [86] Witteman, A.M., Akkerdaas, J.H., Van, L.J., van der Zee, J.S. and Aalberse, R.C. (1994) *Int. Arch. Allergy Immunol.*, 105, 56-61.
- [87] Ayuso, R., Reese, G., Leong-Kee, S., Plante, M. and Lehrer, S.B. (2002) *Int. Arch. Allergy Immunol.*, 129, 38-48.
- [88] Reese, G., Tracey, D., Daul, C.B. and Lehrer, S.B. (1996) *Adv. Exp. Med. Biol.*, 409, 225-230.
- [89] Asturias, J.A., Gomez-Bayon, N., Arilla, M.C., Martinez, A., Palacios, R., Sanchez-Gascon, F. and Martinez, J. (1999) *J. Immunol.*, 162, 4342-4348.
- [90] Kretsinger, R.H. and Nockolds, C.E. (1973) *J. Biol. Chem.*, 248, 3313-3326.
- [91] Messer, N.G. and Kendrick-Jones, J. (1988) *FEBS. Lett.*, 234, 49-52.
- [92] Pomés, A., Smith, A.M., Grégoire, C., Vailes, L.D., Arruda, L.K. and Chapman, M.D. (2001) *Allergy Clin. Immunol. Int.*, 13, 162-169.
- [93] Yang, C.Y., Wu, J.D. and Wu, C.H. (2000) *Biochim. Biophys. Acta.*, 1517, 153-158.

---

Received: October 10, 2006    Revised: April 25, 2007    Accepted: April 28, 2007