Two new types of allergens from the cockroach, *Periplaneta americana*

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**Abstract**

*Periplaneta americana* cockroach is an important source of inhalant indoor allergen resource, and there are more than twenty IgE-binding components identified in *P. americana*, but only nine allergens were characterized. Our knowledge about cockroach allergens remains poor. In this work, two novel allergen proteins Per a 11 (alpha-amylase) and Per a 12 (chitinase) with molecular weight around 55 and 45 kDa, respectively, were purified and characterized from the midgut of cockroaches. Their primary sequences were determined by Edman degradation, mass spectrometry, and cDNA cloning. Sera from 39 and 30 of 47 (83.0% and 63.8%) patients reacted to Per a 11 and Per a 12 on immunoblots, respectively. The allergenicity of Per a 11 and Per a 12 was further confirmed by competitive ELISA, basophil activation test (BAT), and skin prick test (SPT). They appear to be of importance for the allergic reactions induced by cockroach and have a potential for component-based diagnosis of allergy.

Cockroaches can induce IgE-mediated type I hypersensitivity (1, 2) and are related with asthma development, especially among inner-city children (3–5). There are significant differences in sensitization pattern for cockroach among countries and individuals. This discrepancy may result from differences in cockroach source containing variable types and levels of allergens, processing methods, storage conditions, life style of people, etc. Much difficulty is encountered to purify and characterize allergens for highly variable protein content and species in cockroach materials. At least twenty-two immunoglobulin E (IgE)-binding components were identified in *P. americana* (6), but only nine allergens including Per a 1 (enzyme related with digestion) (⁷), Per a 2 (aspartic protease) (⁸), Per a 3 (arylphorin) (⁹), Per a 4 (lipocalin) (¹⁰), Per a 6 (troponin C) (¹¹), Per a 7 (tropomyosin) (¹²), Per a 9 (arginine kinase) (¹³), Per a 10 (serine protease) (¹³), and troponin T were characterized. More than half of American cockroach allergens have not been characterized. Given the
extreme diversity of cockroach allergens, much work about purifying and characterizing novel allergens still needs to be done. This study was aimed to identify novel allergens from *P. americana* by biochemical approaches, SPTs, and immunoblotting.

**Material and methods**

Sera were collected from 47 cockroach-allergic patients (24 males and 23 females; range, 8–67 years; median, 28 years) who had a positive SPT to crude *P. americana* extract. Additionally, sera from 10 healthy individuals who showed negative SPT responses to crude *P. americana* extract were collected as negative controls. The study was approved by the ethics committee of Kunming Institute of Zoology, Chinese Academy of Sciences. Written informed consent was obtained from all participants.

The midguts of the cockroaches were excised, homogenized, and centrifuged, and the supernatant was termed CME (cockroach midgut extract). Allergens from CME were purified by size-exclusion chromatography, anionic exchange column as illustrated in Fig. 1. Their primary sequences were determined by Edman degradation, mass spectrometry, and cDNA cloning. Immunoblotting analysis, competitive inhibition ELISA, BAT, and SPT were performed to determine the allergic potency of the purified proteins according to previously described method (14, 15).

For additional supporting information about material and methods, see Supplementary document.

**Results**

As illustrated in Fig. 1, two allergens were purified from CME by gel filtration and anionic exchange column. The purified proteins as indicated by arrows in Fig. 1C–D were analyzed by SDS-PAGE, revealing that they are homogeneous proteins with molecular weight of 55 and 45 kDa (inserts in Fig. 1C–D).

The amino acid sequences of N-terminus and partial interior peptide fragments of these two purified proteins were obtained by Edman degradation and mass spectrometry analysis (Fig. S3). These sequences were found to share high similarity with *Blattella germanica* allergen alpha-amylase (Bla g 11) and Dermatophagoides farinae allergen chitinase (Der f 15), respectively. Therefore, these two new allergens were named Per a 11 and Per a 12. cDNAs encoding their precursors (the accession numbers of Per a 11 and Per a 12 in GenBank are KR019685 and KR019686, respectively) were cloned from the cDNA library of *P. americana* midgut. The precursor of Per a 11 and Per a 12 is composed of 494 and 407 aa, respectively, as illustrated in Fig. S1.

To determine the allergenicity of these two purified proteins, immunoblotting was performed using individual sera from 47 cockroach-allergic patients, and it demonstrated that sera IgE from 39 (83.0%) and 30 (63.8%) of 47 cockroach-allergic patients reacted to Per a 11 and Per a 12, respectively. IgE-binding ability of these two purified allergens in a representative group of seven patients and two controls is illustrated in Fig. 2. For ELISA inhibition assays, two patients’ sera which

![Figure 1](https://via.placeholder.com/150)
showed positive reactions to both Per a 11 and Per a 12 were chosen. The maximal inhibition of patients’ IgE antibodies binding to the coated CME by Per a 11 and Per a 12 was about 60% and 40%, respectively. For in vitro basophil activation test, both allergens demonstrated capacity to induce IgE cross-linking in patients’ basophils. In comparison with healthy control, Per a 11 and Per a 12 at 1.0 μg/ml induced approximately up to 5.8- and fivefold increase, respectively, in the number of CD63 and CCR3 double-positive cells following incubating them with peripheral blood mononuclear cells from patients with cockroach allergy (Fig. S2). In addition, skin prick testing showed that 12 (80%) and 9 (60%) of 15 cockroach-allergic patients had positive SPT reactions to Per a 11 and Per a 12, respectively (Fig. S4 and Table S1).

Discussion

Cockroach allergy is a widespread health problem in the world, mainly associated with the development of asthma (16, 17). Cockroach allergens consist of a wide group of proteins with diverse structures and biological functions (6). However, only nine American cockroach allergens have been identified so far, and seven of them have been added to the WHO/IUIS Allergen Nomenclature database (http://www.allergen.org/). In this work, two new major allergens (Per a 11 and Per a 12) are purified and characterized from the midgut of cockroaches. Per a 11 is an alpha-amylase with a 77% amino acid sequence identity to Bla g 11 identified from German cockroach. Per a 12 is a chitinase with a 33% amino acid sequence identity to Der f 15 and Der p 15 identified from dust mite. These two novel allergens Per a 11 and Per a 12 have been officially approved by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee.

Alpha-amylase (EC 3.2.1.1) is a ubiquitous endoglycosidase that hydrolyze alpha-linked polysaccharides to yield glucose and maltose. Alpha-amylase was identified as a common allergen in many species including from bacteria (Bacillus licheniformis, Bac li aA), yeast (Saccharomyces cerevisiae, Sac c glucosidase), fungus (Aspergillus oryzae, Asp o 2), barley (Hordeum sativum, Hor v 16) to insects (B. germanica, Bla g 11; D. pteronyssinus, Der f 4; Aedes aegypti, Aed a 4; Culicoides nubeculosus, Cul n 8). Sequence alignment of alpha-amylases from different species revealed that American cockroach alpha-amylase Per a 11 shares the highest identity with B. germanica amylase Bla g 11 (77.3% identity) (Fig. S1B), followed by mite group 4 allergens (Der p 4, 44.61%; Blo t 4, 44.07%; Eur m 4, 42.65%). Per a 11 was found to share low similarity with allergenic amylases from other insects, bacteria, yeast, fungus, and barley (11.62–15.95%), whereas fungal and barley alpha-amylase are an important occupational allergen for baker in the bakery industry. Presently, it is unknown whether there is a cross-reactivity between alpha-amylases from different species. Recently, a potential
conserved IgE-binding epitope for the cross-reactivity of mite group 4 allergens was described (18). Therefore, it is necessary to investigate the cross-reactivity between cockroach amylase and other allergenic amylases from different species.

Chitinase (EC 3.2.1.14) can hydrolyze the N-acetyl-D-glucosamine 1,4-β-linkages of chitin polymers and is essential for the digestion of chitin-containing nutrients in mite and cockroach gastrointestinal tract. Dust mite chitinases (Der f 15 and Der p 15) are known as clinically important allergens (19, 20). So far, no native chitinase allergen has been identified from arthropods. A chitinase (Per a 12) was purified and characterized from the midgut of the cockroaches and provided direct proof that there is allergenic chitinase in arthropods. Per a 12 shows high sequence identity (33%) with mite chitinases Der f 15 and Der p 15 (Fig. S1D).

Allergenicity assessment of Per a 11 and Per a 12 revealed that they are major allergens of American cockroach, which reacted to sera IgE from 80% and 60% patients with cockroach allergy, respectively, using immunoblots and SPT (Fig. 2, Fig. S4 and Table S1). ELISA inhibition assays indicated that maximal inhibition by Per a 11 and Per a 12 was about 60% and 40% (Fig. 2), respectively. So together, they would account for about 100% inhibition, and this would demonstrate that there are not many more allergens accounting for the IgE binding to the CME for the two patients whose sera were used for the ELISA inhibition. This of course does not exclude the possibility that other patients are sensitive to other American cockroach allergens. In fact, immunoblotting of adult Periplaneta americana whole body extract showed that there are at least twenty-two IgE-binding components with pooled cockroach-allergic patients’ sera (6). Thus, it is possible that there are other allergens in American cockroach body, or in their feces, saliva, shed skins, and egg cases.

The identification of two new P. americana allergens will be helpful for cockroach allergy diagnosis and therapy, especially for patients without response for other cockroach allergens. In addition, the current work extended the repertoire of P. americana allergens although continuous cockroach allergen identification is necessary.

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Author contributions

R. L. and S. A. designed the project, supervised the experiments and wrote the manuscript. Other authors performed the experiments and revised the manuscript.

Conflict of interest

The authors report no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

- **Figure S1.** cDNA encoding alpha-amylase (Per a 11, GenBank accession number KR019685) (A) and chitinase (Per a 12, GenBank accession number KR019686) (C) allergens and their amino acid sequences comparison with German cockroach allergen Bla g 11 (B) and dust mite allergen Der f 15 (D). Per a 11 shares a 77% amino acid sequence identity with Bla g 11 (B) and Per a 12 shares a 33% sequence identity with Der f 15 (D). The amino acid sequences of peptide fragments determined by Edman degradation and mass spectrometry are underlined, and the predicted signal sequence is in italic; *, stop condon; The accession numbers of Bla g 11 and Der f 15 in GenBank are ABC68516.1 and AF178772.1, respectively. The identical residues are marked with Consensus.

- **Figure S2.** Induction of basophil activation by Per a 11 and Per a 12. Allergen was incubated with six cockroach-allergic patients’ PBMC (A1, Per a 11; B1, Per a 12) or six healthy subjects’ PBMC (A2, Per a 11; B2, Per a 12). NC, using the stimulation buffer to evaluate the basal CD63 level.

- **Figure S3.** (A) Partial interior peptide fragments of Per a 11 were determined by ESI-QUAD-TOF mass spectrometry. (B) Partial interior peptide fragments of Per a 12 were determined by ESI-QUAD-TOF mass spectrometry.

- **Figure S4.** Representative results of SPTs.

- **Table S1.** Results of skin prick tests.

- **Data S1.** Material and methods.

References

8. Pan QR, Wang SM, Shang HS, Chew FT. Identification and characterization of Per a 2, the Bla g 2 allergen homologue from American cockroach (*Periplaneta americana*).


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