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# Characterization of an anti-Bla g 1 scFv: Epitope mapping and cross-reactivity

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ABSTRACT

Bla g 1 is a major allergen from *Blatella germanica* and one of the primary allergens used to assess cockroach allergen exposure. The epitope of an anti-Bla g 1 scFv was mapped in order to better understand cross reactivity with other group 1 cockroach allergens and patient IgE epitopes. X-ray crystallography was used to determine the structure of the scFv. The scFv epitope on Bla g 1 was located by alanine scanning site-directed mutagenesis and ELISA. Twenty-six rBla g 1-GST alanine mutants were evaluated for variations in binding to the scFv compared to the wild type allergen. Six mutants showed a significant difference in scFv binding affinity. These mutations clustered to form a discontinuous epitope mainly comprising two helices of Bla g 1. The allergen-scFv complex was modeled based on the results, and the epitope region was found to have low sequence similarity with Per a 1, especially among the residues identified as functionally important for the scFv binding to Bla g 1. Indeed, the scFv failed to bind Per a 1 in American cockroach extract. The scFv was unable to inhibit the binding of IgE antibodies from a highly cockroach allergic patient to Bla g 1. Based on the surface area of Bla g 1 occluded by the scFv, putative regions of patient IgE–Bla g 1 interactions can be inferred. This scFv could be best utilized as a capture antibody in an IgE detection ELISA, or to differentiate Bla g 1 from Per a 1 in environmental exposure assays.

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## 1. Introduction

Allergic sensitization to cockroach-derived molecules is a risk factor for the development of asthma (Matsui et al., 2010; Salo et al., 2008). There are currently ten groups of cockroach allergens to which patients commonly become sensitized (www.allergen.org). In comparison to cat or dust mite for which a few allergens dominate most of the IgE response, the IgE response to cockroach allergens can be highly variable (Pomés et al., 2007). The three most commonly recognized allergens produced by the cockroach *Blattella germanica* are Bla g 1, Bla g 2, and Bla g 5, but the prevalence of IgE in patients in the U.S. is only 26%, 54%, and 37%, respectively (Satinover et al., 2005).

Bla g 1 and Bla g 2 are the most commonly used allergens for the assessment of cockroach allergen exposure. The threshold dose of Bla g 1 exposure established as a risk factor for sensitization is 2 U/g of dust, and 8 U/g is considered to be a risk factor for asthma morbidity (Eggleston et al., 1998; Rosenstreich et al., 1997). Allergen levels are commonly measured with antibodies raised against cockroach extracts (Pollart et al., 1991a). The cockroach extracts used to standardize these assays were initially assigned an arbitrary value based on a fixed volume of extract (Pollart et al., 1991b). The amount of Bla g 2 in 1 Unit was determined to be 80 ng, subsequent to cloning and characterization (Arruda et al., 1995; Gustchina et al., 2005). Whereas Bla g 2 is a stable globular protein, Bla g 1 is a more complex allergen, and has a fragmentation pattern on SDS-PAGE that made standardization difficult for a long time. It was only recently that 1 Unit of the allergen Bla g 1 was standardized to be 104 ng (Mueller et al., 2013). This will facilitate a better comparison of allergen exposure levels. The need for strict molecular standards instead of arbitrary units is best reflected in a study of 6 commercial cockroach extracts in which there was up to a 200 fold difference in the Bla g 1 levels (4.7–1085 U/ml) (Patterson and Slater, 2002).

Bla g 1 is a unique allergen that is composed of multiple tandem repeats of two distantly related core sequences termed α and β (Helm et al., 1996; Pomés et al., 1998; Randall et al., 2013). In other

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insect species, up to 7 copies of α and β are present on a single polypeptide chain (Randall et al., 2013). The two core sequences each form a pentagon of alpha helices with a sixth helix displaced above the plane of the pentagon (Mueller et al., 2013). The two pentagons of α and β interact via the rim, creating a large internal hydrophobic cavity that can bind various lipids (Mueller et al., 2013). The unstructured loops between α and β are frequently proteolyzed, leading to the mistaken impression on SDS-PAGE analysis that the protein is highly fragmented and therefore there is a consequent loss of antibody epitopes. It has been demonstrated that even with variable fragmentation patterns, antibody recognition of the allergen was consistent, indicating that the core structure remains intact (Mueller et al., 2013).

In order to better understand antibody epitopes on Bla g 1, we sought to characterize the interaction between an avian derived scFv and recombinant Bla g 1 (deVore et al., 2010; Finlay et al., 2005; Khurana and Slater, 2013). This particular scFv is proposed to be part of a multiplex assay that is under development to study the composition and potency of *B. germanica* extracts used in clinical settings. Knowledge of the particular epitope may be useful in understanding the cross-reactivity of the scFv with other cockroach species allergens, and may be useful in mapping patient IgE epitopes.

## 2. Materials and methods

### 2.1. Structure determination

The anti-Bla g 1 scFv was expressed in *E. coli* as a maltose binding protein (MBP) fusion (pDEST vector 566, provided to the NIEHS Protein Expression Core Facility by Dominic Esposito, SAIC, NCI Maryland), purified by amylose affinity chromatography followed by removal of the His-tagged-MBP by cleavage with TEV protease. The His-tag facilitated removal of the MBP and TEV with a Nickel column. For ELISA experiments, the scFv was further purified by size exclusion chromatography. For crystallography, the scFv was combined with rBla g 1-EC purified as previously described (Mueller et al., 2013), followed by size exclusion chromatography. The eluted protein corresponding to the scFv–Bla g 1 complex was concentrated to 30 mg/ml in 25 mM Tris pH 7.5 and 75 mM NaCl for crystallization screening. Crystals of the complex were not obtained, but crystals of the scFv alone were obtained by hanging drop vapor diffusion in a mother liquor of 1.8 M ammonium sulfate, 100 mM NaCl, 50 mM Tris pH 7.5–8.5 at 20 °C. Glycerol was added as a cryo-protectant at 15% (v/v), and the crystals were flash frozen in liquid $N_2$. The data were collected at the Southeast Regional Collaborative Access Team (SER-CAT) 22 ID beamline at Advanced Photon Source and were processed with HKL2000 (Otwinowski and Minor, 1997). The structure was solved by molecular replacement using the coordinates of an anti-interferon scFv (3UX9) (Ouyang et al., 2012) as the starting model, followed by multiple rounds of model building and refinement in COOT and PHENIX (Emsley and Cowtan, 2004; Zwart et al., 2008). Data collection and refinement statistics are presented in Table 1. The final model contains scFv residues Q1-L107 and A115-V231.

### 2.2. Alanine scanning mutagenesis and ELISA

Thirty alanine mutants of rBla g 1-EC (Table 2) were created by site-directed mutagenesis using QuikChange II (Agilent). Twenty-six constructs expressed sufficient soluble protein for further testing. The mutants were purified by glutathione affinity and judged better than 95% pure by SDS-PAGE. The GST-tag was not removed for the ELISA experiments.

ELISA plates were coated overnight at 4 °C with 10 μg/ml scFv in coating buffer: 0.84% $NaHCO_3$ (w/v), 0.356% $Na_2CO_3$ (w/v), pH

**Table 1**
Crystallographic data table.

| | |
|---|---|
| Unit cell | $a = 102.5$ Å, $b = 51.1$ Å, $c = 44.9$ Å<br>$\alpha = \gamma = 90°$ $\beta = 105.0$ |
| Space group | C2 |
| Resolution (Å) | 50.0–1.8 |
| No. of observations | 66,691 |
| Unique reflections | 19,780 |
| $R_{sym}$ (%) (last shell)[a] | 4.8 (17.6) |
| $I/\sigma I$ (last shell) | 18.1 (4.5) |
| Mosaicity range | 0.39–0.59 |
| Completeness (%) (last shell) | 94.6 (72.0) |
| Refinement statistics | |
| $R_{work}$ (%)[b] | 16.5 |
| $R_{free}$ (%)[c] | 19.1 |
| No. of waters | 157 |
| Overall mean *B* value (Å) | |
| Protein | 27.5 |
| Solvent | 37.9 |
| r.m.s. Deviation from ideal values | |
| Bond length (Å) | 0.011 |
| Bond angle (°) | 1.36 |
| Dihedral angle (°) | 11.5 |
| Ramachandran statistics[d] | |
| Residues in: | |
| Favored (98%) regions (%) | 97.4 |
| Allowed (>99.8%) regions (%) | 100 |

[a] $R_{sym} = \sum (|I_i - \langle I \rangle|) / \sum (I_i)$ where $I_i$ is the intensity of the *i*th observation and <*I*> is the mean intensity of the reflection.
[b] $R_{work} = \sum ||F_o| - |F_c|| / \sum |F_o|$ calculated from working data set.
[c] $R_{free}$ was calculated from 5% of data randomly chosen not to be included in refinement.
[d] Ramachandran results were determined by MolProbity.

**Table 2**
Bla g 1 mutatations and $\Delta\Delta G$ compared to wild type measured by ELISA.

| Mutation | $\Delta\Delta G$ |
|---|---|
| Q41A | −0.05 |
| V49A | * |
| D50A | 0.06 |
| I53A | 0.09 |
| T57A | * |
| L60A | 0.00 |
| D76A | −1.10 |
| E79A | −1.20 |
| T80A | −1.70 |
| V83A | −0.33 |
| N93A | 0.31 |
| N96A | 0.33 |
| N104A | 0.40 |
| D107A | 0.13 |
| H113A | 0.09 |
| H129A | −0.38 |
| V133A | −0.08 |
| D141A | * |
| D151A | 0.15 |
| E161A | 0.33 |
| L167A | * |
| N171A | −0.14 |
| E177A | −0.11 |
| Q183A | 0.33 |
| T184A | −0.25 |
| E190A | −0.62 |
| N193A | −2.10 |
| K197A | −1.40 |
| E200A | −0.02 |
| D206A | −0.01 |

* Failed to express.