

Molecular Structure of Cockroach Allergens

M.D. Chapman^a L.D. Vailes^a V. Dhanaraj^b T.L. Blundell^b A. Pomés^a

^aAsthma and Allergic Diseases Center, University of Virginia, Charlottesville, Va., USA;

^bDepartment of Biochemistry, University of Cambridge, Cambridge, UK

Key Words

Cockroach allergen · Asthma · Aspartic proteinase

Introduction

Cockroaches produce potent allergens that are an important cause of asthma among patients living in cockroach-infested housing. We have cloned and expressed a series of allergens from German cockroach (*Blattella germanica*), and report molecular studies of the structures of Bla g 1 and Bla g 2.

Bla g 1 is a cross-reactive allergen with approximately 70% sequence identity to Per a 1 from *Periplaneta americana*. A novel feature of these allergens is that they comprise multiple tandem repeats of approximately 100 amino acid residues and appear to be derived from an approximately 90-kD precursor. Sequence analysis reveals a number of trypsin cleavage sites and homology to mosquito ANG12 protein, suggesting that the allergen is produced in the gut and is cleaved into multiple molecular forms of different molecular weights. These proteins also show homology to mitochondrial energy transfer proteins and may have evolved through gene duplication [1]. Bla g 2 is a major cockroach allergen that shows homology to aspartic proteinases [2], and it has been expressed at high

levels in *Pichia pastoris*. Since proteolytic activity has been suggested to contribute to the induction of IgE responses, we investigated whether Bla g 2 has functional enzymatic activity.

Here we report high-level expression of Bla g 1 constructs containing 2 or 4 amino acid repeats in *Escherichia coli* and/or *P. pastoris*. We investigated the reactivity of different recombinant (r)Bla g 1, and compared the IgE antibody binding to natural and rBla g 1. We also obtained rBla g 2, and compared IgE antibody binding to natural and recombinant allergen. We assessed the importance of enzymatic activity for the allergenicity of Bla g 2 by molecular modeling of Bla g 2 and by studying its enzymatic activity and examining critical residues in the catalytic site that are responsible for enzymatic activity.

Materials and Methods

Several rBla g 1 constructs containing 2 or 4 repeats were expressed in *E. coli* using the pET 22b(+) and the pET 21d(+) vectors and inserting Bla g 1.01 DNA in the *Nco* I and *Xho* I sites. A leader sequence was expressed using the pET 22b(+) vector. Bla g 1 was also expressed in *P. pastoris*. A construct containing 4 repeats was subcloned into the pPICZaB vector using the restriction sites *Pst* I and *Not* I. The vector, linearized using *Bst*X I, was electroporated into *Pichia*. Seven transformants Mut^s were obtained from the KM17 strain grown on media containing 500 µg/ml zeocin, one of which secreted high levels of rBla g 1 after methanol induction (10–20 mg/

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2001 S. Karger AG, Basel

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. Anna Pomés
Asthma and Allergic Diseases Center, Department of Medicine
UVa Health System, PO Box 801355
Charlottesville, VA 22908-1355 (USA)
Tel. +1 804 982 3328, Fax +1 804 924 5779, E-Mail nkm8t@virginia.edu



Fig. 1. Model of the Bla g 2 three-dimensional structure showing the α -helices (cylinders) and β -sheets (arrows) of the typical bilobed aspartic proteinase shape. The two coplanar aspartates in the catalytic site and the phenylalanine 75 in the 'flap' are also shown.

liter). Expressed rBla g 1 was purified by affinity chromatography over a 10A6 monoclonal antibody column, followed by size exclusion chromatography using a Superdex column and an FPLC system. Purified rBla g 1 showed reactivity in a quantitative ELISA that measures Bla g 1 units [3]. IgE antibody binding to affinity-purified rBla g 1 was also measured by an ELISA that uses a chimeric mouse/human antibody to Der p 2 to construct the IgE binding standard curve [4].

A model of the cockroach allergen Bla g 2 was constructed by the rule-based comparative modelling approach using the software Modeller, based on the high-resolution crystal structures of the homologous pepsin and chymosin. Enzymatic activity was measured using the milk clotting assay described previously [5]. Pepsin and chymosin activity was used as the standard. Serial dilutions of samples (20 μ l) were incubated in plate wells with 12% w/v dried milk in 0.2 M sodium acetate, pH 5.4, and 10 mM CaCl_2 (80 μ l) for 1 h at 37°C. Pepstatin and PMSF were used as inhibitors of aspartic and serine proteinase activities, respectively.

Results

Bla g 1

Several constructs of recombinant Bla g 1.01 containing 2 or 4 repeats were expressed in *E. coli* and *P. pastoris*. Limited reactivity in ELISA was shown by the constructs expressed in *E. coli*, and attempts to refold the insoluble proteins did not improve their reactivity. The constructs expressed in *Pichia* retained the correct folding necessary for antibody binding. Bla g 1 expressed by *P. pastoris* was purified and showed the same activity as natural Bla g 1 in a monoclonal antibody ELISA assay. A good correlation

was found between the binding of IgE antibodies from sera of 34 Bla g 1 allergic patients to rBla g 1 expressed in *P. pastoris* and IgE binding to the natural allergen ($r = 94$; $p < 0.001$).

Bla g 2

Bla g 2 was expressed in *P. pastoris*. A good correlation was found between the binding of IgE antibodies from sera of 26 Bla g 2 allergic patients to rBla g 2 expressed in *P. pastoris* and IgE binding to the natural allergen ($r = 93$; $p < 0.001$).

Molecular modeling showed that the overall three-dimensional structure of Bla g 2 is similar to that of aspartic proteinases (fig. 1). Bla g 2 has an unusually high number of cysteins (11), compared to the three pairs present in most aspartic proteinases. Only two of these three pairs are present in Bla g 2. Comparative analysis suggests that Bla g 2 has a well-defined binding pocket. However, critical amino acid substitutions in the catalytic triads (DST and DTS instead of DTG) and in some residues of the pocket (e.g. Phe75 instead of Tyr) indicate that Bla g 2 is inactive. Bla g 2 is related to a group of mammalian pregnancy-associated glycoproteins found in cattle and sheep which also have inactive catalytic triads and are thought to serve as binding proteins. In keeping with this, Bla g 2 had no activity in the milk clotting assay for aspartic proteinase activity. These results suggest that functional proteinase activity does not account for the allergenic activity of Bla g 2.

Discussion

In summary, a eukaryotic expression system was used to express rBla g 1 and rBla g 2 that bind IgE from cockroach-allergic patients in the same way as the natural allergens. These recombinant allergens will be used to study the diagnosis of cockroach allergy by skin tests in cockroach-sensitized individuals, and to perform crystallographic studies of the three-dimensional structure of Bla g 1 and Bla g 2. The results define structurally diverse features of cockroach allergens, and indicate that the induction of IgE responses to Bla g 2 is not determined by enzymatic activity and that this allergen may function as a binding protein.

Acknowledgment

This study was supported by the NIH grants AI 32557 and AI 34607.

References

- 1 Pomés A, Melén E, Vailes LD, Retief JD, Arruda LK, Chapman MD: Novel allergen structures with tandem amino acid repeats derived from German and American cockroach. *J Biol Chem* 1998;273:30801–30807.
- 2 Arruda LK, Vailes LD, Mann BJ, Shannon J, Fox JW, Vedvick TS, Hayden ML, Chapman MD: Molecular cloning of a major cockroach (*Blattella germanica*) allergen, Bla g 2. Sequence homology to the aspartic proteases. *J Biol Chem* 1995;270:19563–19568.
- 3 Pollart SM, Smith TF, Morris EC, Gelber LE, Platts-Mills TAE, Chapman MD: Environmental exposure to cockroach allergens: Analysis with monoclonal antibody-based enzyme immunoassays. *J Allergy Clin Immunol* 1991;87:505–510.
- 4 Schuurman J, Perdok GJ, Lourens TE, Parren PWHI, Chapman MD, Aalberse RC: Production of a mouse/human chimeric IgE monoclonal antibody to the house dust mite allergen Der p 2 and its use for the absolute quantification of allergen-specific IgE. *J Allergy Clin Immunol* 1997;99:545–550.
- 5 Nugent PG, Albert A, Orprayoon P, Wilsher J, Pitts JE, Blundell TL, Dhanaraj V: Protein engineering loops in aspartic proteinases: Site-directed mutagenesis, biochemical characterization and X-ray analysis of chymosin with a replaced loop from rhizopuspepsin. *Protein Eng* 1996;9:885–893.

Copyright: S. Karger AG, Basel 2001. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.