Affinity-Purified Vitellin/Vitellogenin, a Major Component of Total Allergen Recognized by German Cockroach Specific IgE

Christian Hadiwinarta[1], Alexander Blank[1], Michael B. Strader, PhD[1], Kavita Sharma, PhD[1], Robert G. Hamilton, PhD[2], Coby Schal, PhD[3], Charles Bronzert[2], Jay E. Slater, MD[1]

1. Laboratory of Immunobiochemistry, Division of Bacterial, Parasitic, and Allergenic Products, Office of Vaccines Research and Review, CBER/FDA, Silver Spring, MD, USA
2. Division of Allergy and Clinical Immunology, Department of Medicine, and Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, 21205, USA
3. Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA

Abstract

The German cockroach (GCr) generates aeroallergens associated with allergic rhinitis and asthma. Vitellogenin (Vg) / vitellin (Vn), a high molecular mass and an abundant protein in GCr hemolymph, eggs, and egg cases, is a novel candidate allergen. Prior efforts to purify Vg/Vn, yielded amounts too small for allergen characterization experiments. In this study, we adopted an affinity purification strategy using a rabbit anti-Vg column to obtain high yields of purified vitellogenin from whole body defatted GCr powder. This strategy resulted in 91% pure Vg/Vn, as determined by LC-MS/MS analysis. To further characterized Vg/Vn as an allergen, ImmunoCAP (Thermofisher Scientific/Phadia) assays were performed to study direct binding and inhibition using specific antibodies sera from GCr sensitized individuals. Vg/Vn inhibited IgE binding to ImmunoCAP(I6) in 3 sera in a dose-dependent manner over a range of added Vg/Vn (5-42.5µg), with maximal inhibition of 30-54%. In conclusion, purified Vg/Vn demonstrated that Vg/Vn minor but common German cockroach allergen which contributes towards GCr sensitization.

Introduction

German cockroach (GCr; *Blattella germanica*) is an important source of indoor allergens associated with allergic rhinitis and asthma. GCr allergen extracts are not standardized and have not been characterized for allergen protein content. In our initial proteomic screen of GCr allergen extracts and source materials, we identify vitellogenin/vitellin (Vg/Vn) as a candidate allergen that appears to be present in large amounts in crude GCr extracts. In previous work we isolated Vg/Vn from GCr oothecae (egg cases), but yields were limited by available source material and too low for our subsequent research needs. In this work we purify Vg/Vn from commercially available crude GCr powder by affinity chromatography using a rabbit anti-Vg/Vn serum.

Materials and Methods

Crude allergen extract was prepared from GCr powder (Greer) in ammonium bicarbonate buffer (pH 8, 1:10, w/v) shaken overnight at 4°C and filtered with Whatman #1 filter paper. Protease inhibitors (Roche) were added to all buffers used prior to purification. Anti-Vg/Vn serum was raised in New Zealand white rabbits by intradermal injection of electrophoretically isolated Vg/Vn with Freund's complete and incomplete adjuvants (Tufail et al. 2000). IgG was purified from the sera by protein A chromatography (Thermo Scientific) and conjugated to cyanogen bromide-activated Sepharose 4B (Cytiva Sweden AB). The filtered allergen extract was then passed over the anti-Vg/Vn Sepharose, and the Vg/Vn was eluted with glycine buffer (0.05M, pH 2.5). The eluate was resolved by SDS-PAGE and characterized by liquid-chromatography high-resolution mass spectrometry (LC-HRMS) using a LUMOS Tribid Orbitrap mass spectrometer. The relevance of Vg/Vn as a GCr allergen was assessed in two ways. First, GCr-allergic sera were preincubated with Vg/Vn and inhibition of IgE binding to GCr-ImmunoCAP (I6) was calculated. Second, direct binding of IgE form GCr-allergic individuals to Vg/Vn was assessed using a streptavidine-ImmunoCAP preincubated with biotinylated Vg/Vn.

Materials and Methods

Affinity Purification of Vg/Vn

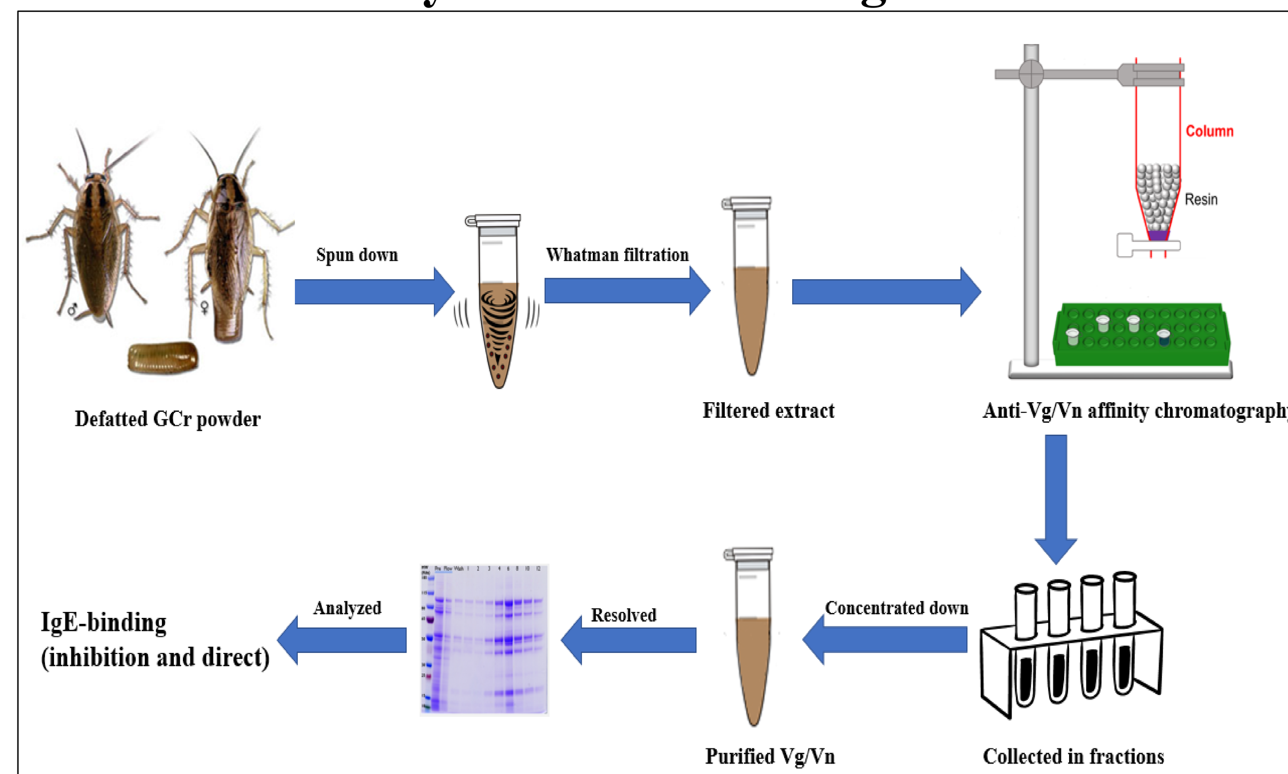
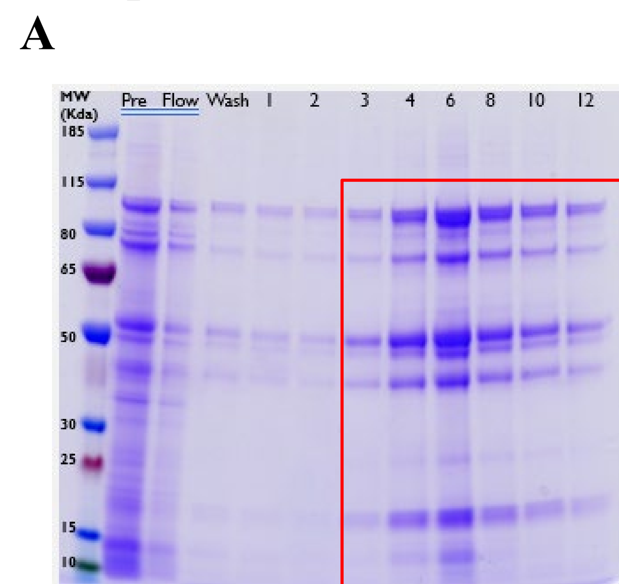


Figure 1. Crude GCr extract was prepared from defatted GCr powder, and the centrifuged and filtered extract was passed over an anti-Vg/Vn affinity column. The column was washed extensively, and the putative Vg/Vn was eluted at pH 2.5 and neutralized.

Results and Discussion

Low pH Elution Fractions A



Proteomic Analysis of Vg/Vn B

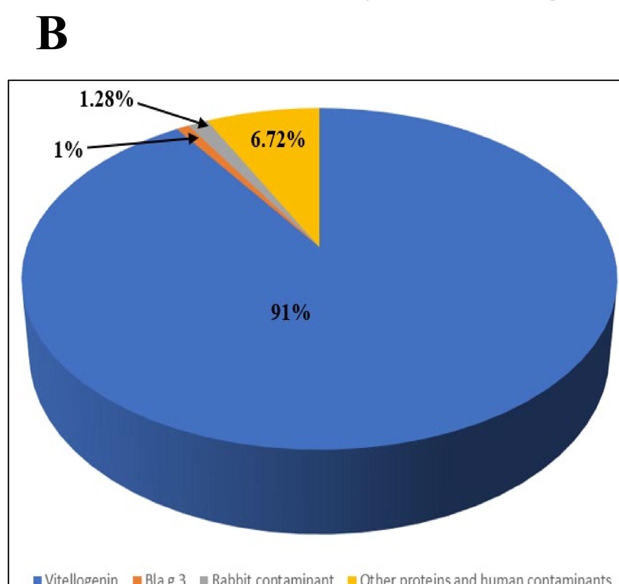


Figure 2. (A) SDS-PAGE analysis of eluates from affinity purification of Vg/Vn. Fractions 3 through 12 were collected for further analysis. (B) Proteomic analysis of pooled Vg/Vn fraction by LC-HRMS. Of the analyzed sequences, 91% map of the total measured ion current was mapped to Vg/Vn peptides. Other eluate components included Bla g 3 (1%), rabbit contaminants (1.28%), and other proteins, including human contaminants (6.72%).

Results and Discussion

Dose-Response of Vg/Vn

Serum Identifier	Buffer (kUA/L)	+Vg/Vn (kUA/L)				Inhibition (%)			
		1µg	5µg	20µg	42.5µg	1µg	5µg	20µg	42.5µg
U9313	20.8	17.9	16.7	12.4	9.59	13.9	19.7	40.4	53.9
U9378	19	18.3	18.6	15.2	13.3	3.7	2.1	20	30
V1984	20	16.8	17.6	14.7	10.9	16	12	26.5	45.5

Table 1. Vg/Vn inhibition of IgE binding to GCr (I6). Serum from GCr-allergic individuals was preincubated with Vg/Vn and binding to ImmunoCAP(I6) was determined. For all three sera maximal inhibition (30 - 54%) was noted at 42.5 µg.

Identification of IgE Anti-Vg/Vn in GCr-sensitized Sera

Serum Identifier	Original analysis (kUA/L)	+Vg/Vn (kUA/L)	+Buffer (kUA/L)	Inhibition (%)
U3056	71.8	75.3	78.4	4
U3171	73.9	79.2	85.5	7.4
U3520	13.5	10.8	11.9	9.2
U6769	19.3	23.3	24.7	5.7
U6821	27.7	16.7	18.5	9.7
U8772	11.6	17	16.6	-2.4
U9313	33	20.8	27.2	23.5
U9378	25.6	20	25.2	20.6
V1984	20.3	20.3	23.8	14.7
V2491	13.6	13	14	7.1

Table 2. Vg/Vn inhibition of IgE binding to GCr (I6). Serum from GCr-allergic individuals was preincubated with Vg/Vn (5 µg) and binding to ImmunoCAP(I6) was determined. Inhibition >5% was considered significant. Of the 10 sera tested, 8 met this threshold.

Christian Hadiwinarta was supported by an appointment to the Research Participation Program at the Center for Biologics Evaluation and Research administered by the Oak Ridge Institute for Science Education through an interagency agreement between the US Department of Energy and the US Food and Drug Administration.

Direct IgE-binding GCr (I6) vs Vg/Vn

Serum Identifier	GCr(i6-CAP) (kUA/L)	Vg/Vn-CAP (kUA/L)	Vg/Vn / GCr(i6-CAP) ratio (%)
U9313	30.2	1.06	3.50
U9378	25.9	1.2	4.60
U9989	11.7	0.25	2.10
V1984	22.9	0.81	3.50
V4247	8.8	0.19	2.10
V4497	6.5	0.82	12.60
V5141	9.9	0.34	3.40
V5740	9.9	0.26	2.60
V5763	8.4	0.88	10.50
V6236	12.1	0.37	3.10
V6366	7.2	0.42	5.90
V6461	41.8	2	4.80
V6804	6.5	<0.1	ND
U6848	2.64	<0.1	ND
V6848	0.71	<0.1	ND
V6849	11.5	0.36	3.10
V6860	1.94	0.15	7.70
V6876	4.56	0.19	4.20
V7069	1.07	0.21	19.60
V7085	1.83	<0.1	ND
V7133	1.04	0.2	19.20
V7212	7.24	1.23	17.00
V7245	1.35	0.12	8.90
V7267	9.18	0.7	7.60
V7271	1.26	<0.1	ND
V7303	1.03	0.28	27.20

Table 3. Direct binding of IgE from GCr-allergic individuals to GCr extract (I6-CAP) ImmunoCAP-avidin coated with biotinylated Vg/Vn. Specific IgE to Vg/Vn was detected in 21/26 sera at the detectable threshold of >0.10 kUA/L, and in 11/26 sera at >0.35 kUA/L. * ND, Not Detected.

Conclusion

- Affinity purification strategy using a rabbit anti-Vg/Vn column resulted in high yields of purified vitellogenin from whole body defatted GCr powder.
- Purification of Vg/Vn was determined by LC-HRMS to 91%.
- Direct binding assay to biotinylated Vg/Vn showed that 81% (21 of 26) GCr-allergic sera had detectable specific IgE; 42% (11/26) GCr-allergic sera had specific IgE above the 0.35 kUA/L threshold.
- The IgE anti-Vg/Vn concentration ranges from 2-27.2% to the total IgE anti-GCr.

References

Tufail M, Lee JM, Hatakeyama M, Oishi K Takeda M. Cloning of vitellogenin cDNA of the American cockroach, *Periplaneta americana* (Dictyoptera), and its structural and expression analysis. Arch Insect Bioch Physiol, 45:37–46 (2000).

Research supported by Intramural FDA CBER funds