

Cloning, Expression, and Immunological Characterization of Formosan Subterranean Termite (Blattodea: Rhinotermitidae) Arginine Kinase

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Abstract

Several parts of the world regularly consume termites. Arthropod arginine kinase proteins often cross-react with human immunoglobulin E (IgE) antibodies and they are considered pan-allergens. The Formosan subterranean termite *Coptotermes formosanus* (*C. formosanus* [Shiraki] [Isoptera: Rhinotermitidae]), along with cockroaches, belong to the order Blattodea and they are common household pests in tropical and subtropical parts of the world. An sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) band migrating at approximately 37 kDa in *C. formosanus* termite extracts cross-reacted with IgE from five cockroach allergic patient samples by immunoblot. Liquid chromatography–mass spectrometry analysis of gel slices from the corresponding region of a gel indicated several peptides from the excised region were identical to the American cockroach arginine kinase allergen, Per a 9. The sequence of the full-length *C. formosanus* arginine kinase gene indicates the protein it encodes is 96% identical to American cockroach Per a 9, 94% identical to German cockroach Bla g 9, and 82–84% identical to shrimp arginine kinase proteins Pen m 2, Lit v 2, and Cra c 2. Full-length *C. formosanus* arginine kinase was fused to a glutathione S-transferase tag and recombinantly expressed and purified from *Escherichia coli* by affinity chromatography. The recombinant protein was recognized by IgE from 11 of 12 cockroach or shrimp allergic samples, but did not cross-react with dust mite allergic or peanut/tree nut allergic samples. The results of this study indicate the *C. formosanus* arginine kinase cross-reacts with cockroach and shrimp allergic IgE, and if consumed would likely act as an allergen.

Key words: termite, cockroach, arginine kinase, IgE, cross-react

The increasing incidence of food allergy and asthma results in substantial health, psychological, and economic burdens (Liu et al. 2010, Gupta et al. 2013). Estimates indicate that 32 million people (11% of adults and 8% of children) in the United States suffer from food allergies (Gupta et al. 2018, 2019). The Global Asthma Report of 2018 estimates the global burden of disease for asthma is 339.4 million people, with asthma affecting approximately 40 million individuals in the United States (Varghese and Asher 2018). Common food allergy triggers include eggs, milk, wheat, soy, peanuts, tree nuts, fish, and shellfish while airborne allergens include pollens, molds, animal dander, and insects, such as cockroaches (Plasek et al. 2016). Food allergic reactions are a result of an inappropriate

immune response via immunoglobulin E (IgE) binding to food proteins.

As the use of insects as food increases, proteins from edible insects such as termites and cockroaches will likely pose a serious allergy risk (Ribeiro et al. 2018). Cockroaches are an important source of insect allergens, and termites and cockroaches are closely related within the order Blattodea (Inward et al. 2007, Pomés et al. 2017). Termites and cockroaches are significant pests that live in urban centers within human dwellings, and termite colonies are made of millions of insects. Home infestations can bring humans into direct contact with termites, potentially allergenic frass (termite droppings), mud tubes for travel, and nests. During mating season, millions of winged reproductive

termites (alates) are attracted to light and may have human contact as the insects fly around looking for suitable nesting sites. Further, discarded alate wings can accumulate inside human dwellings in the thousands each year after swarming and remain for years as they breakdown and become part of airborne household dust.

Arginine kinase is a major allergen in cockroaches, house dust mites, and shellfish, and is a pan-allergen (Sookrung et al. 2003, 2006). Cockroach arginine kinase is a 40–45 kDa protein with multiple IgE-binding sites that cross-react with IgE from shrimp allergic patients (Sookrung et al. 2006, Chuang et al. 2010). Shrimp, other shellfish, and seafood arginine kinases are abundant proteins and are important contributors to shellfish allergy (Lopata et al. 2010, 2016; Shen et al. 2012; Chen et al. 2013; Faber et al. 2017).

Extracts from the Formosan subterranean termite, *Coptotermes formosanus*, Shiraki (Blattodea: Rhinotermitidae) have been demonstrated to cross-react with cockroach-specific antibodies and to compete for IgE binding in serum from cockroach allergic individuals (Mattison et al. 2017). Further, recombinant *C. formosanus* tropomyosin (Copt f 7) was recognized by serum IgE from cockroach and shellfish allergic patients and induced basophil degranulation at concentrations comparable to shrimp tropomyosin (Vargas et al. 2018). Identifying and characterizing potential new allergens or characterizing very similar proteins that do not commonly act as allergens could help identify factors that contribute to allergic disease. Due to the close evolutionary relationship and the high degree of protein sequence similarity, the objective of this research was to determine if termite arginine kinase could cross-react with cockroach IgE arginine kinase antibodies. Here, using cockroach and shrimp allergic volunteer samples an IgE reactive immunoblot band is correlated with termite arginine kinase, and recombinant arginine kinase protein produced in *Escherichia coli* is shown to be recognized by IgE.

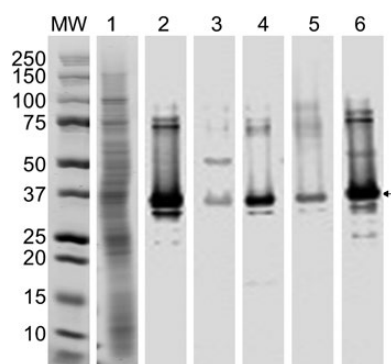


Fig. 1. Recognition of a 35–37 kDa band in defatted whole termite extract by IgE from five cockroach allergic volunteers. SDS-PAGE (lane 1) and IgE immunoblot of whole termite extract with five cockroach allergic volunteer samples (lanes 2–6). Arrow indicates migration position of 35–37 kDa band. Molecular weight markers are shown in the left-most panel.

Materials and Methods

Materials

Novex 10–20% Tricine and Tris-glycine sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and iBlot gel transfer stacks were from Life Technologies (Carlsbad, CA). Termites were collected from traps located in New Orleans City Park (New Orleans, LA) and defatted protein extracts were collected as described in Mattison et al. (2017). Samples were collected from long-standing Formosan termite colonies, and species identity was verified by mitochondrial 16S ribosomal DNA sequencing (Szalanski et al. 2003). Secondary goat anti-rat IRDye 800CW was purchased from LI-COR (Lincoln, NE). Allergic volunteer serum/plasma samples were collected using informed written consent from volunteer donors (IRBNet#: 410953-1) at the San Antonio Uniformed Services Health Education Consortium (SAUSHEC) Allergy/Immunology (Lackland AFB, TX) or were purchased from PlasmaLab International (Everett, WA). Characteristics of volunteer samples including the results of clinical diagnostic ImmunoCAP allergen-specific IgE blood tests are described in Supp Table 1 (online only). The hybridoma clone 38G6 secreting a Per a 9 monoclonal antibody (MAb38G6) has been characterized previously (Sookrung et al. 2003, 2006).

Recombinant Arginine Kinase Production

A codon-optimized reading frame of *C. formosanus* arginine kinase was synthesized by IDT (Coralville, IA) with flanking 5'BamHI and 3'EcoRI restriction sites and cloned into the pGEX-6P1 expression vector (GE Healthcare Life Sciences, Pittsburgh, PA) to make plasmid CPM555. *Escherichia coli* BL21 (DE3) cells were transformed with the CPM555 plasmid and cells were grown in Luria Broth with 100 µg/ml ampicillin to an optical density at 600_{nm} of 0.4. Arginine kinase expression was induced by the addition of 1 mM isopropyl-thio-β-galactoside, and cells were grown for 4 h. Cells were harvested by centrifugation and the collected cells were lysed by sonication in 100 mM sodium phosphate buffer (pH 7.4) containing 250 mM NaCl, 0.1% triton X-100, 1 mM dithiothreitol (DTT), and 1 mM phenylmethylsulfonyl fluoride (PMSF). Cell lysates were centrifuged and the recombinant arginine kinase was purified from the clarified lysate by affinity chromatography with Glutathione Agarose (Pierce-Thermo Fisher Scientific, Waltham, MA) according to manufacturers' instruction. The recombinant arginine kinase was cleaved from the glutathione beads by incubation with PreScission protease (GE Healthcare, Pittsburgh, PA) overnight at 4°C. Liberated protein was buffer exchanged into phosphate-buffered saline (PBS) by dialysis and then stored at –80°C.

LC-MS/MS Mass Spectrometry

Mass-spectrometric analysis of SDS-PAGE gel slices and recombinant *C. formosanus* arginine kinase was performed essentially as described by Mattison et al. (2014). Bands of unknown proteins were excised from SDS-PAGE gel and digested with trypsin, or in the case of purified recombinant arginine kinase digested directly after purification,

Table 1. Peptides from 35–37 kDa region of termite extract matching American cockroach arginine kinase (Per a 9) sequence

Protein name	Species	Peptide start amino acid	Peptide sequence	m/z measure (Da)	Z	MH+ matched (Da)	MH+ error (ppm)
Arginine kinase	<i>C. formosanus</i> /P. americana	165	(K)GQFYPLTGMTK(E)	621.814	2	1,242.62	1.8
Arginine kinase	<i>C. formosanus</i> /P. americana	230	(R)IISMQMGGDLGQVYR(R)	834.41	2	1,667.82	–7.2
Arginine kinase	<i>C. formosanus</i> /P. americana	265	(R)LGFLTFCPTNLGTTVR(A)	898.974	2	1,739.92	2.2
Arginine kinase	<i>C. formosanus</i> /P. americana	295	(K)AKLEEYAGKYNLQVR(G)	430.243	4	1,717.96	–5.9
Arginine kinase	<i>C. formosanus</i> /P. americana	297	(K)LEEYAGKYNLQVR(G)	506.949	3	1,518.83	3.7

ATG GTG GAT CAA GCA GTG CTG GAC AAG CTA GAG GCG GGA TAC GCC AAG TTG GCT GCC TCT GAC AGC
M V D Q A V L D K L E A G Y A K L A A S D S
 AAG TCT CTG CTC AAG AAA TTT TTA ACC AAG GAA GTA TTT GAC AAC CTC AAG ACC AAG AAG ACC CCA
 K S L L K K F L T K E V F D N L K T K K T P
 TCT TTC GGC TCT TCG CTT CTT GAT GTA ATC CAA TCC GGT CTT GAG AAC CAC GAC TCT GGA GTC GGT
 S F G S S L L D V I Q S G L E N H D S G V G
 ATC TAC GCG CCA GAT GCG GAG GCT TAC TCT GTG TTT GCT GAT CTG TTT GAC CCC ATC ATT GAG GAC
 I Y A P D A E A Y S V F A D L F D P I I E D
 TAC CAT GGT GGC TTC AAG AAG ACC GAT AAG CAT CCA CCA AAG GAC TGG GGT GAT GTG GAC ACT CTG
 Y H G G F K K T D K H P P K D W G D V D T L
 GGC AAC CTG GAC CCT GCT GGC GAG TAC ATC ATC TCG ACA AGA GTG CGG TGT GGT CGC TCA CTG GAG
G N L D P A G E Y I I S T R V R C G R S L E
 GGC TAC CCC TTC AAT CCC TGT CTG ACC GAA GCA CAG TAC AAG GAG ATG GAG GAG AAG GTC TCC AGC
G Y P F N P C L T E A Q Y K E M E E K V S S
 ACG CTG TCT GGT CTG GAG GGT GAG CTG AAG GGT CAG TTC TAC CCT CTC ACT GGC ATG ACC AAG GAA
T L S G L E G E L K G Q F Y P L T G M T K E
 GTT CAG CAG AAG CTG ATT GAT GAC CAC TTC CTG TTC AAG GAA GGT GAT CGC TTC CTG CAG GCT GCC
 V Q Q K L I D D H F L F K E G D R F L Q A A
 AAT GCA TGC CGC TTC TGG CCC ACT GGA CGA GGC ATA TAC CAC AAC GAT GCC AAG ACG TTC CTG GTA
N A C R F W P T G R G I Y H N D A K T F L V
 TGG TGC AAT GAG GAG GAT CAT TTG CGA ATC ATC TCC ATG CAG ATG GGG GGT GAC CTA GGA CAG GTA
W C N E E D H L R I I S M Q M G G D L G Q V
 TAC CGT CGT CTT GTG ACA GCT GTG AAT GAT ATT GAG AAG CGC ATT CCT TTC TCG CAT GAT GAC CGC
Y R R L V T A V N D I E K R I P F S H D D R
 CTG GGC TTC CTC ACC TTC TGC CCT ACC AAC CTG GGT ACC ACA GTG CGT GCA TCA GTG CAC ATC AAG
L G F L T F C P T N L G T T V R A S V H I K
 GTA CCC AAG CTG GCT GCT GAC AGG GCC AAG CTA GAG GAG GTT GCT GGC AAA TAC AAC TTG CAG GTC
 V P K L A A D R A K L E E V A G K Y N L Q V
 CGT GGG ACA CGC GGT GAA CAC ACA GAA GCC GAG GGT GGT GTG TAT GAC ATC TCC AAC AAG CGG CGT
R G T R G E H T E A E G G V Y D I S N K R R
 ATG GGC CTC ACA GAA TAC GAA GCT GTG AAG GAG ATG CAT GAT GGC ATT GCC GAG CTC ATC AAG CTA
M G L T E Y E A V K E M H D G I A E L I K L
 GAG AGC TCC CTC TAA
 E S S L *

Fig. 2. Nucleotide and deduced amino acid sequence of *C. formosanus* arginine kinase. Underlined residues were observed by LC-MS/MS in tryptic digests of the recombinant protein, italicized residues correspond to Pen m 2-cell epitopes, and bolded residues correspond to the flexible loop involved in substrate specificity.

and analyzed via LC with MS/MS (LC/MS/MS) by an Agilent 1200 LC system (Agilent Technologies, Santa Clara, CA). The system was fitted with an Agilent Chip Cube interface and an Agilent 6520 Q-TOF

tandem mass spectrometer. Mass spectra were analyzed with MassHunter software (Agilent Technologies), and Spectrum Mill software (Agilent Technologies) for protein identification.

Immunoblotting

Protein samples (20 µg of defatted whole termite extract or 1 µg of purified recombinant protein) subjected to SDS-PAGE were transferred to PVDF membrane for 7 min using an iBlot instrument and iBlot gel transfer stacks (Life Technologies, Carlsbad, CA). Blots were blocked with 2% dry milk in PBS (pH 7.4) containing 0.1% Tween-20 (PBST) for 1 h, and incubated with cockroach allergic volunteer samples (diluted 1:5 in PBST) or the MAb38G monoclonal antibody (diluted 1:500 in PBST) overnight at 4°C. Blots were washed three times in 5 ml of PBST with gentle rocking, and the appropriate secondary antibody, mouse anti-human IgE biotin-labeled (1:5,000 in PBST) (Southern Biotech, Birmingham, AL) or donkey anti-mouse IRDye 800CW antibody (1:10,000 in PBST) was added. The blots were incubated for 1 h at room temperature. For visualization of IgE binding, blots were washed three times with 5 ml of PBST, and then incubated with IRDye 680RD-labeled streptavidin (diluted 1:10,000 in PBST). Following three final washes with 5 ml of PBST, blots were scanned and visualized with using an Odyssey imaging system (LI-COR).

Enzyme-Linked Immunosorbent Assay

Purified recombinant arginine kinase (1 µg) was added to plate wells in 50 µl of sodium carbonate (SC) buffer (0.015 M Na₂CO₃, 0.035 M NaHCO₃, pH 9.6) and used to coat plate wells overnight at 4°C. Plate wells were washed three times with PBS (10 mM phosphate, 137 mM sodium chloride, pH 7.4) and blocked the next morning with SC buffer containing 0.1% BSA for 1 h at 37°C. After washing three times with PBS, 50 µl of allergic volunteer sample diluted 1:5 in PBS was added to the wells and incubated for 1 h at 37°C. Following sample removal, plate wells were washed three times with 100 µl of PBST, and 50 µl of biotinylated anti-human IgE (Southern Biotech) diluted 1:1,000 was added to the wells. Following a 1-h incubation at 37°C, wells were washed three times with 100 µl PBST and 50 µl of streptavidin (LI-COR) diluted 1:5,000 was added to each well. After three more 100 µl PBST washes, the IgE signal was documented by scanning the plates with an Odyssey CLX imaging system (LI-COR). Plotted data represent the average of at least four experimental repetitions with standard deviation as error bars.

Protein Modeling

A molecular model of *C. formosanus* arginine kinase was rendered using the I-TASSER server (Yang and Zhang 2015). The model was based upon the crystal structure of the Atlantic horseshoe crab arginine kinase (Azzi et al. 2004). Visualization and highlighting of protein regions was done with Chimera software (Yang et al. 2012).

Table 2. IUIS arginine kinase allergen homology to termite arginine kinase

Allergen	Accession	% Identity	% Similarity
Per a 9	ACA00204.1	96	98
Bla g 9	ABC86902.1	94	98
Plo i 1	Q95PM9	87	94
Bomb m 1	Q2F5T5	87	94
Pen m 2	C7E3T4	83	91
Lit v 2	Q004B5	82	91
Cra c 2	D7F1J5	82	91
Der f 20	AIO08850.1	75	85
Der p 20	B2ZSY4	75	84

Results

Cockroach Allergic IgE Binding to *C. formosanus* Termite Extract Proteins

While characterizing proteins in *C. formosanus* extracts with samples from cockroach allergic individuals, a band migrating near the 37 kDa marker, approximately 35–37 kDa, was observed by immunoblot in five samples (Fig. 1). Mass-spectrometric analysis of gel slices from the corresponding region of an SDS-PAGE gel from the same extracts identified several peptides that matched exactly to those from the American cockroach arginine kinase allergen, Per a 9 (Table 1).

Coptotermes formosanus Arginine Kinase Gene Sequence

Invertebrate arginine kinase proteins are considered pan-allergens and previous work has identified gene sequence from the Formosan subterranean termite (*C. formosanus*) that is homologous to characterized cockroach and shrimp arginine kinase allergens (Vargas et al. 2018). Close inspection of a draft *C. formosanus* genome identified a full-length clone of the termite arginine kinase. The full-length gene encodes a predicted protein of 356 amino acids with an expected mass of 39kDa (Fig. 2). The termite arginine kinase characterized in this paper has significant homology to other IUIS-recognized arginine kinase allergens including Per a 9, Bla g 9, Pen m 2, Lit v 2, and Cra c 2 (Table 2).

Recombinant *C. formosanus* Arginine Kinase Protein Purification

A synthetic codon-optimized *C. formosanus* arginine kinase gene was synthesized and subcloned into a pGEX expression vector, creating an amino-terminal fusion to glutathione S-transferase. The resulting fusion protein had a predicted molecular weight of 65 kDa. Induction of the transformed *E. coli* strain harboring the CPM555 plasmid with IPTG demonstrated the presence of an inducible band migrating between the 75 and 50 kDa markers after 1 h that increased in intensity at 4 h of induction (Fig. 3A). Isolated protein was obtained with glutathione sepharose beads and cleavage with Prescission protease (Fig. 3B). A sample of the purified protein was digested with trypsin and analyzed by mass spectrometry, and 22 distinct peptides (45% total protein coverage) were matched to the Per a 9 arginine kinase confirming the identity of the purified recombinant protein. The purified recombinant protein was recognized by IgE from a pool of five cockroach allergic volunteers and by the MAb38G anti-Per a 9 monoclonal antibody, although recognition by MAb38G was relatively weaker (Fig. 3C).

Cross-reaction of IgE Toward Recombinant *C. formosanus* Arginine Kinase

IgE recognition of the recombinant termite arginine kinase from a panel of 12 cockroach, dust mite, and/or shrimp allergic individuals (Supp Table 1 [online only]) was evaluated by enzyme-linked immunosorbent assay (ELISA). IgE binding to the recombinant termite arginine kinase from 9 of the 12 donor samples (#1–9) that had elevated cockroach, shrimp, and/or dust mite IgE was readily observed (Fig. 4). However, three of the test samples with elevated cockroach, shrimp, and/or dust mite IgE (#10–12) were only mildly reactive. In contrast, primarily dust mite allergic samples (#14–21) and two control samples from tree nut allergic volunteers (samples #13 and 22) did not appreciably recognize the recombinant arginine kinase.

Coptotermes formosanus Arginine Kinase Model

To visualize the protein, a model of the *C. formosanus* arginine kinase was generated. The predicted *C. formosanus* arginine kinase structure contains 10 alpha-helical and 10 beta-strand segments interspersed with coiled segments (Fig. 5). Residues corresponding

to the flexible loop involved in substrate binding based upon a conserved domain search (Marchler-Bauer et al. 2017) are highlighted in green (Fig. 5E) and residues corresponding to Pen m 2 T-cell epitopes in blue (Fig. 5F) (Renand et al. 2014). Comparison of linear IgE epitopes mapped to the Lit v 2 white shrimp arginine kinase (Ayuso et al. 2012) with the corresponding sequence of *C. formosanus* arginine kinase indicated several stretches of identical amino acids (Fig. 6A). Modeling these onto the termite arginine kinase demonstrates a large amount of surface area is covered by these IgE epitopes (Fig. 6B). Antigenic residues identified as important for conformational epitopes in arginine kinase from crab (D3, K33, T174, and W204) were conserved in the termite arginine kinase (Yang et al. 2015) (Fig. 6A and C). Nearby amino acids surrounding these residues likely contribute to the formation of important conformational epitopes (Fig. 6C). The conserved spacing of these antigenically important residues on the structure of termite arginine kinase is striking, and reinforces the conservation among these proteins.

Discussion

The incidence of food allergy has increased over the past decade and as consumption of insects gains popularity awareness of potential allergens must increase. The findings presented here strongly support the conclusion that termite arginine kinase could act as a food allergen. Termite arginine kinase peptides were observed in a band migrating near 37 kDa that was recognized by IgE from cockroach and shrimp allergic volunteers. We cannot exclude that there were other proteins in that region of the gel that were also recognized by IgE. For example, termite tropomyosin has been demonstrated to cross-react with IgE from cockroach and shrimp allergic volunteers and would be expected to migrate

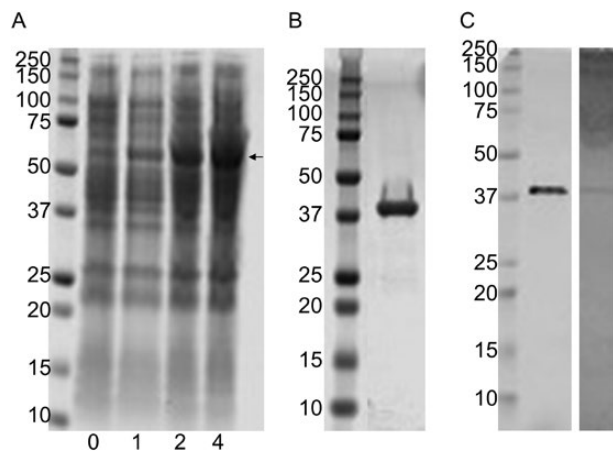


Fig. 3. Expression and purification of recombinant termite arginine kinase. Recombinant termite arginine kinase was expressed as a fusion to glutathione S-transferase (GST) (panel A, arrow), isolated with Glutathione Agarose, and purified by cleavage with Prescission protease (panel B). Induction of the recombinant *C. formosanus* arginine kinase after 1, 2, and 4 h is shown in panel A. The purified protein is recognized by IgE from a pool of five cockroach allergic volunteer samples (left panel C) and the MAb38G monoclonal antibody directed toward American cockroach arginine kinase Per a 9 (right panel C). Molecular weight markers are shown to the left of each panel.

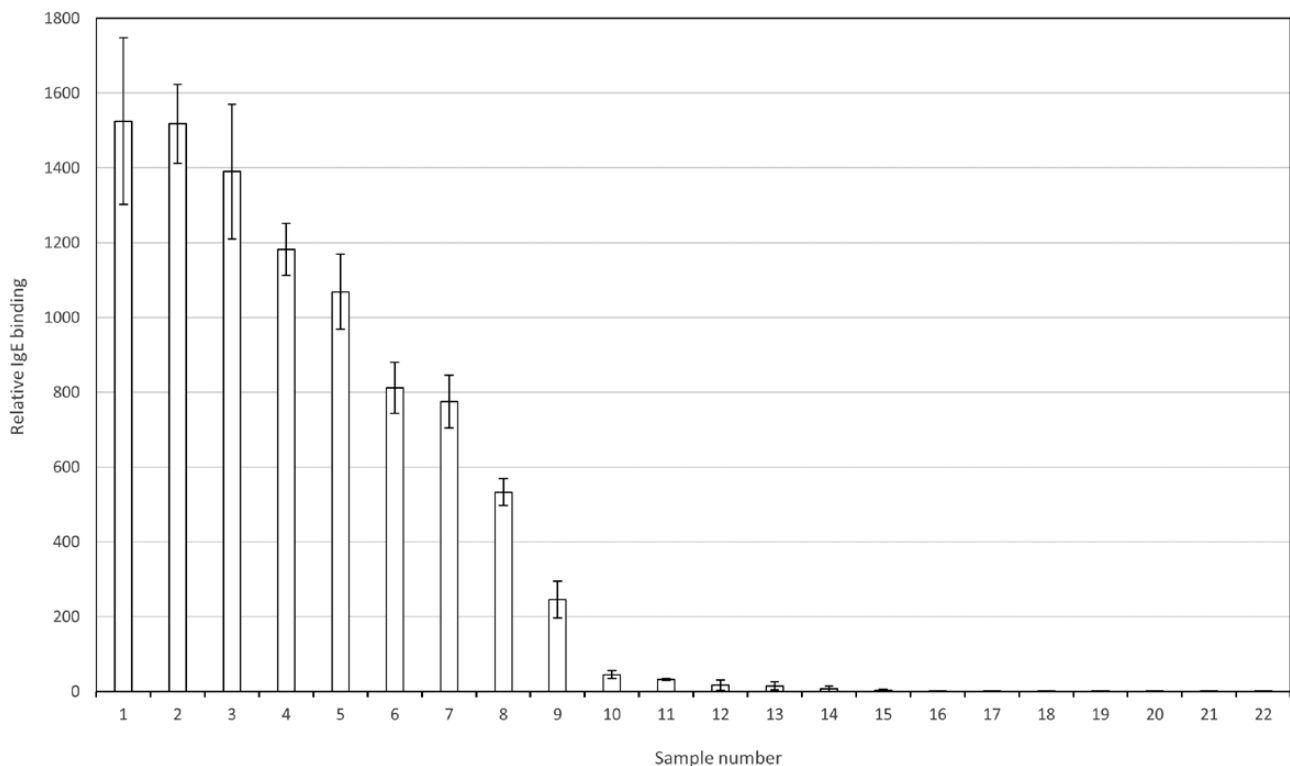


Fig. 4. Direct ELISA measurement of IgE binding to recombinant *C. formosanus* arginine kinase by a panel of cockroach, dust mite, and shrimp allergic volunteer samples. Data represent the average of at least four sample replicates and error bars indicate standard deviation.

arginine kinase may pose a threat to those with shellfish, cockroach, or other arthropod allergies due to cross-reactivity, and may potentially contribute as an allergenic sensitizing agent in geographic areas infested with termites.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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Supplementary Table S1. Characteristics of Individual Volunteer Samples*

Sample number	cockroach	shrimp	dust mite Df	dust mite Dp	peanut	cashew	pecan	soy
1	24.57	61.19	-	21.47	<0.35	<0.35	<0.35	<0.35
2	>100.00	>100.00	-	-	-	-	-	-
3	>100.00	>100.00	>100.00	84.7	41	10.6	2.22	4.34
4	66.5	84.3	-	-	-	-	-	-
5	17.13	-	-	22.44	25.12	-	-	19.95
6	26	7.98	-	<0.35	<0.35	<0.35	<0.35	<0.35
7	79.5	-	46.9	66.1	-	-	-	-
8	33.7	39.8	92.2	90.1	-	-	-	-
9	40.63	34.98	-	19.69	<0.35	<0.35	<0.35	<0.35
10	11.07	17.54	-	43.53	<0.35	<0.35	<0.35	<0.35
11	1.43	2.76	-	54.83	52.45	41.66	23.72	6.23
12	0.76	0.5	5.66	4.24	<0.35	<0.35	<0.35	<0.35
13	-	<0.35	-	<0.35	<0.35	33.6	-	-
14	<0.35	<0.35	0.7	1.2	1.21	<0.35	<0.35	0.47
15	<0.35	<0.35	7.62	7.03	1.95	<0.35	<0.35	<0.35
16	<0.35	<0.35	6.99	6.68	<0.35	<0.35	<0.35	<0.35
17	<0.35	<0.35	1.19	0.83	<0.35	<0.35	<0.35	<0.35
18	<0.35	<0.35	0.5	0.41	<0.35	<0.35	<0.35	<0.35
19	<0.35	<0.35	6.57	7.09	<0.35	<0.35	<0.35	<0.35
20	<0.35	<0.35	1.66	1.31	<0.35	<0.35	<0.35	<0.35
21	<0.35	<0.35	0.46	0.42	0.55	<0.35	<0.35	0.99
22	-	-	-	-	-	28	7.29	-

*Values represent the results of clinical ImmunoCAP diagnostic testing for human IgE to specific allergen sources