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# Identification and analysis of a conserved immunoglobulin E-binding epitope in soybean G1a and G2a and peanut Ara h 3 glycinins

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**Abstract**

To identify conserved immunoglobulin E (IgE)-binding epitopes among legume glycinins, we utilized recombinant soybean G2a and G2a-derived polypeptide fragments. All of these fusion polypeptides bound IgE, and the C-terminal 94-residue fragment appeared to bind more IgE. Using synthetic peptides we identified S219–N233 ($S^{219}$GFAPEFLKEAFGVN$^{233}$) as the dominant IgE-binding epitope. Alanine scanning of this epitope indicated that six amino acids (E224, F225, L226, F230, G231, and V232) contributed most to IgE binding. Among these amino acids, only G231 of soybean G2a is not conserved in soybean G1a (S234) and peanut Ara h 3 (Q256). Synthetic peptides corresponding to the equivalent regions in G1a and Ara h 3 bound IgE in the order Ara h 3 ≥ soybean G2a > soybean G1a. This sequence represents a new IgE-binding epitope that occurs in a highly conserved region present in legume glycinins. Such IgE-binding sites could provide a molecular explanation for the IgE cross-reactivity observed between soybean and peanut proteins.

**Keywords:** Soybean allergens, Peanut, Recombinant proteins, IgE cross-reactivity, Epitope mapping, Conserved epitope, Alanine scanning

**Abbreviations:** IgE, immunoglobulin E; G1a, glycinin G1 acidic chain; G2a, glycinin G2 acidic chain; SDS, sodium dodecyl sulfate; PCR, polymerase chain reaction; TBST, Tris-buffered saline-Tween 20; HRP, horseradish peroxidase.

Food allergic hypersensitive responses are mediated through immunoglobulin E (IgE)[1] in allergic individuals. Soybean and peanut can cause human allergic reactions and rank among the eight most significant food allergens [1, 2]. They both belong to the legume family and share high similarity in many of their seed storage proteins [3]. In vitro IgE cross-reactions between soybean and peanut are not uncommon. Serum samples from patients allergic to soybean or peanut proteins often contain IgE that can react with seed proteins from either species [4–8]. The occurrence of peanut allergy is more frequent in western countries and the responses are frequently severe. Allergic reactions to peanut are often life-long and occur in both children and adults. Conversely, soybean allergic reactions can be transient, occurring more often in children and normally do not cause severe allergic responses [3]. It had been shown that the cross-reactions between soybean and peanut proteins might not be clinically significant [6–9]. However, a recent study indicated that soybean proteins might be underestimated as a cause of severe allergic response in the general population of peanut-allergic patients [10]. This finding, as well as increased reports of soybean allergy linked to the greater use of soybean protein in the food industry [2], raises new concerns for soybean allergy and its impact on people with known or latent allergies to peanut [10]. It is evident that we need careful biochemical and immunological studies to evaluate IgE-binding proteins in both species, with a goal of identifying homologous epitopes that can potentially elicit serious allergic reactions [3].

Our studies have focused on the soybean glycinins because of their abundance (>20% of seed dry weight) in soybean seeds and their high sequence similarity with other legume family storage proteins. Native glycinins are hexamers of 350 kDa, and each subunit is composed of a 40-kDa acidic and a 20-kDa basic chain that are linked through interchain disulfides [11, 12]. Both chains of soybean glycinin proteins bind IgE from soybean allergic patients [13, 14]. Soybean glycinins have high sequence similarity with peanut Ara h 3 [15, 16]. Epitope mapping for IgE-binding has been performed for glycinin G1 acidic chain (G1a), glycinin G2 acidic chain (G2a), and Ara h 3 in previous studies, and there were some sequence similarities in some of these identified epitopes [16–18]. However, in these studies sera from patients with known peanut allergies were used in the epitope mapping of Ara h 3 and sera from patients with known soybean allergies were used to identify G1a or G2a epitopes. In our current study, we have focused on finding conserved IgE-binding epitopes among these three related glycinins. The same serum, containing IgE interacting with both soybean and peanut, was used for discovering a conserved IgE-binding epitope in the glycinins.

## Materials and methods

*Construction, expression, and purification of recombinant fusion proteins of glycinin G1a and G2a.* Recombinant G1a and G2a were generated and purified as thioredoxin-fusion proteins using the pBAD/TOPO expression system following manufacturer-supplied protocols (Invitrogen) as described previously [17].

*Construction of recombinant proteins of glycinin G2a fragments.* Two recombinant proteins representing the N-terminal, G2af1, and the C-terminal, G2af2, fragments of G2a were constructed. The PCR primers designed for the G2af1 were G2aBADL (50-gcccata tgagaga gcaggc) and G2a208R*Hin*dIII (5′-aagcttttg ttgctttcct ttc); the PCR primers designed for G2af2 were G2a203L*Nde*I (5′-catatgcagaaaggaaagcaac) and G2a296R*Hin*dIII (5′-aagcttttt-gctttggcg). All primers were synthesized by Sigma–Genosys (The Woodlands, TX). PCR reactions were performed for 30 cycles using *Taq* polymerase (Gibco-BRL). PCR products were purified from 1% agarose gel and ligated into the PCR2.1–TOPO vector using the TA system (Invitrogen). The ligated DNA was transformed into *Escherichia coli* Top10 cells following manufacturer recommended protocols (Invitrogen). Constructs containing inserts with the predicted sizes were sequenced in the Genomics Core Research Facility of the University of Nebraska–Lincoln. Sequence-verified inserts were subsequently obtained by double digestions using *Nde*I and *Hin*dIII restriction endonucleases and ligated into the pET28a vector (Novagen).

*Expression and purification of recombinant proteins.* The pET28a plasmids containing sequence-verified inserts were transformed into *E. coli* BL21 (DE3) cells. *E. coli* containing either the G2af1 or the G2af2 construct were grown in 5 ml LB medium containing kanamycin (50 $\mu$g/ml) at 37 °C overnight and used to inoculate 250 ml LB medium containing kanamycin (50 lg/ml). Cultures were grown at 37 °C with shaking at 200× rpm until the $A_{600}$ reached approximately 0.5. At that time, cells were induced with 0.5 mM isopropyl β-D-thiogalactopyranoside. Cells were grown for an additional 3 h at 37 °C and harvested by centrifugation at 5000g for 5min at 4 °C. Purification of the recombinant proteins was as described by Beardslee et al. [17]. Fusion protein purity was evaluated by SDS– PAGE as described below. Protein concentrations were analyzed by the BCA assay (Pierce). Purified proteins were stored in aliquots at –20 °C until used.

*Electrophoretic separation.* Aliquots of recombinant proteins (2 lg for G1a or G2a, 1 $\mu$g for G2af1 or G2af2) were mixed with an equal volume of SDS–PAGE sample loading buffer (100 mM Tris–HCl, pH 6.8, 4% SDS, 0.2% bromophenol blue, 20% glycerol, and 200 mM dithiothreitol) and heated to 95 °C for 5 min. After cooling, samples were analyzed on 12.5% SDS–PAGE gels [19]. Proteins were stained with 0.1% Coomassie brilliant blue R-250.

*Human serum used for study.* Soybean-allergic serum from an adult female approximately 45 years old was purchased from Plasma-Lab (Everett, WA). This patient had positive skin prick tests to both soybean and peanut. She had a reported history of allergic reactions to many beans and experienced anaphylaxis with peanut. The IgE level of this serum was 192 IU/ml, soybean-specific IgE was 4.0 IU/ml, and peanut-specific IgE was 37.5 IU/ml. One patient with high IgE level (1285 IU/ml) but no reported soybean- or peanut-allergic reactions was used as a negative control.

*Immunoblotting.* For immunoblotting after SDS– PAGE separation, proteins were transferred to PVDF membranes (Millipore, Immobilon-P) in a tank apparatus overnight at 10 V in transfer buffer (10 mM Tris–HCl, 100 mM glycine, 10% methanol) at 4 °C. Membranes were blocked overnight with TBST (25 mM Tris–Cl, pH 7.5, 150 mM NaCl, 0.05% Tween 20), containing 5% nonfat dry milk, and subsequently incubated overnight at 4 °C with human serum diluted 1:20 in blocking buffer. After extensive washes (3 × 30 min) with TBST, membranes were incubated with horseradish peroxidase conjugated to goat anti-human IgE (Bethyl Labs, Montgomery, TX) diluted 1:10, 000 in TBST. Membranes were washed as above with TBST and antigen–antibody complexes were detected by chemiluminescence using the ECL plus reagent (Amersham Pharmacia, Piscataway, NJ) and imaged using Biomax ML film (Kodak). Films

were developed using an automatic developer (Kodak M35A, X-OMAT Processor).

*Peptide synthesis on SPOT membrane.* Individual peptides were synthesized on a modified SPOT cellulose membrane (Sigma–Genosys) containing free hydroxyl groups using Fmoc amino acids according to Frank [20] and manufacturer-supplied protocols (Sigma–Genosys). After the addition of the last amino acid, the cellulose membranes were treated with trifluoroacetic acid/dichloromethane/triisopropylsilane (1:1:0.5) to deblock side-chain protecting groups. The membrane was washed sequentially with dichloromethane, dimethyl formamide, and methanol; dried; and stored at –20 °C until needed or used directly for the IgE-binding assay as described below.

*IgE-binding assay of SPOT membrane.* The SPOT membrane containing the synthetic peptides was washed with TBS and blocked overnight at 4 °C with blocking buffer, TBST containing 5% sucrose and 1 × membrane blocking buffer (Sigma–Genosys). The membrane was incubated overnight at 4 °C with the appropriate human serum diluted 1:2 in blocking buffer, washed in TBST, and incubated with goat anti-human IgE–horseradish peroxidase (Bethyl Labs) diluted 1:5000 in blocking buffer and developed using chemiluminescence as described above.

*Structural analysis of G2a IgE epitope.* The amino acid sequence for glycinin G2 (SwissPort Accesssion, P04405) was submitted for modeling to SWISS-MODEL [21] using the soybean glycinin G1 crystal structure [22] as a template. To view the trimeric structure, the glycinin G1 structure was used. The sequence alignment generated by SWISS-MODEL was used to highlight residues corresponding to the G2a IgE epitope. The Swiss-PdbViewer program was used for model manipulation and 3D rendering was performed with POV-Ray 3.

## Results

*Expression, purification, and IgE-binding ability of recombinant soybean G1a and G2a proteins.* Recombinant G1a and G2a proteins were constructed as thioredoxin fusions and were purified by nickel-affinity chromatography (Figure 1A). The G2a fusion protein apparently displayed IgE binding greater than that of the G1a fusion protein (Figure 1B).

*C-terminal fragment of G2a binds IgE more strongly than N-terminal fragment.* To identify potential epitopes on G2a, we produced two recombinant proteins representing the N-terminal and C-terminal portions of G2a. The G2af1 coded for R20-Q208, the N-terminal 189 amino acids of mature G2a after cleavage of the signal peptide of the first 19 residues. The G2af2 fragment coded for Q203-K296, the C-terminal 94 amino acids of G2a. Both G2a fragments also contained a 6histidine purification tag and were purified using a nickel-affinity column (Figure 2A). When equal amounts of purified recombinant proteins of G2af1 and G2af2 (1 $\mu$g each) were loaded on the SDS–PAGE and transferred to the PVDF membrane, the G2af2 fragment exhibited stronger IgE-binding ability to the serum sample used in this study (Figure 2B). From the calculated intensities of the immunoblots (areas obtained from scans) the IgE binding of G2af2 was estimated to be about four times more than that of G2af1. Based on the approximately twofold difference in the molar amounts loaded on the gels, the G2af2 fragment displayed an apparent twofold greater affinity for IgE binding as compared to the N-terminal G2af1 fragment. Serum from a soybean-tolerant individual and secondary antibody controls did not exhibit IgE binding to the G2a fragments (data not shown). These results indicated that the majority of IgE-binding was to the C-terminal fragment of G2a.

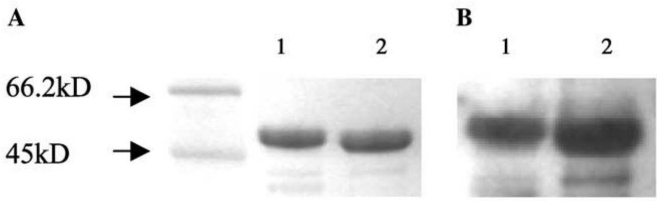



**Figure 1. IgE binding to recombinant G1a protein and G2a protein.** (A) Purified recombinant G1a protein (Lane 1, 2 $\mu$g) and G2a protein (Lane 2, 2 $\mu$g) were separated by 12.5% SDS–PAGE and stained with Coomassie blue. (B) Immunoblotting of recombinant G1a protein (Lane 1, 2 $\mu$g) and G2a protein (Lane 2, 2 $\mu$g) with serum. After SDS– PAGE separation, proteins were transferred to PVDF and immunoblotting was performed as described under Materials and methods. Antibody dilutions were 1:20 for soybean-sensitive sera and 1:10, 000 for goat anti-human IgE–HRP.

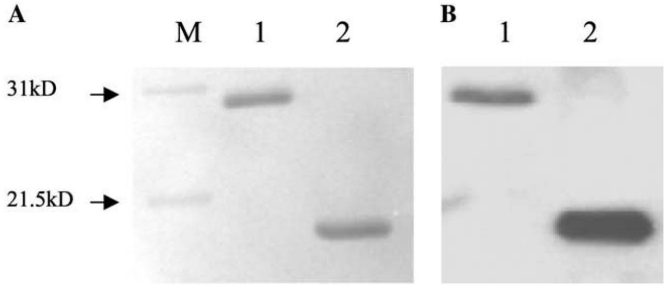
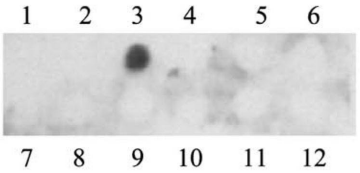


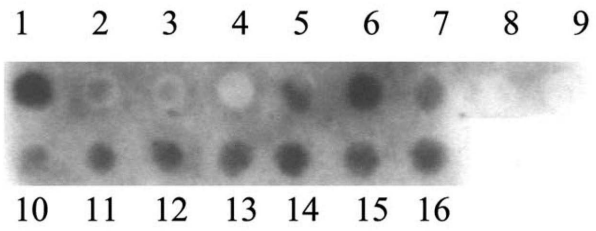

**Figure 2. IgE-binding ability of purified recombinant G2a fragments: G2af1 and G2af2.** (A) Equal amount of purified recombinant G2af1 protein (Lane 1, 1 $\mu$g) and G2af2 protein (Lane 2, 1 $\mu$g) were separated by a 12.5% SDS–PAGE and stained with Coomassie blue. (B) Immunoblotting of recombinant G2af1 protein (Lane 1, 1 $\mu$g) and G2af2 protein (Lane 2, 1 $\mu$g) with serum. After 12.5% SDS–PAGE separation, proteins were transferred to PVDF and immunoblotting was performed as described under Materials and methods. Antibody dilutions were 1:20 for soybean–sensitive sera and 1:10, 000 for goat antihuman IgE-HRP.




| Spot # | Position | Peptide Sequence |
|---|---|---|
| 1 | 205-219 | G K Q Q E E E N E G S N I L S |
| 2 | 212-226 | N E G S N I L S G F A P E F L |
| **3** | **219-233** | **S G F A P E F L K E A F G V N** |
| 4 | 226-240 | L K E A F G V N M Q I V R N L |
| 5 | 233-247 | N M Q I V R N L Q G E N E E E |
| 6 | 240-254 | L Q G E N E E E D S G A I V T |
| 7 | 247-261 | E D S G A I V T V K G G L R V |
| 8 | 254-268 | T V K G G L R V T A P A M R K |
| 9 | 261-275 | V T A P A M R K P Q Q E E D D |
| 10 | 268-282 | K P Q Q E E D D D D E E E Q P |
| 11 | 275-289 | D D D E E E Q P Q C V E T D K |
| 12 | 282-296 | P Q C V E T D K G C Q R Q S K |

**Figure 3. Epitope mapping of G2af2.** Twelve 15-mer peptides representing the whole G2af2 region were manually synthesized on a SPOT membrane as described under Materials and methods. Adjacent peptides had an eight amino acid overlap. The membrane was probed with the soybean sensitive sera (1:2 dilution) and bound IgE was detected by goat anti-human IgE–HRP (1:5000 dilution). The antigen–antibody complexes were detected by chemiluminescence. Peptide 3, which exhibited IgE binding and corresponded to the region of residues S219– N233 of G2a protein, is underlined. The amino acid sequence of this peptide is shown in bold.

*Epitopes in the G2af2 fragment.* We next set out to identify the IgE-binding epitope within the G2af2 fragment. Twelve overlapping 15-mer peptides with eight amino acid overlaps corresponding to the G2af2 sequence were synthesized on modified cellulose paper and probed with the soybean-sensitive serum (Figure 3). Only peptide 3, corresponding to the region S219–N233 of G2a, exhibited strong IgE binding (Figure 3). After longer exposure time, peptide 7, corresponding to E247–V261 of G2a, also showed some IgE binding. IgE binding to these peptides was not detected with control serum (data not shown). To identify the amino acids critical for IgE binding in peptide 3, a series of related peptides were synthesized on a SPOT membrane with alanine substitutions at each position of the 15-mer epitope and their IgE-binding abilities were evaluated (Figure 4). A significant loss of IgE binding was observed after alanine was substituted for F225 (spot 9), L226 (spot 8), and F230 (spot 4). Substitution of G231 (spot 3), V232 (spot 2), and E224 (spot 10) with alanine also resulted in lowered IgE binding. Substitution of alanine at other positions on this peptide did not cause a consistent decrease in IgE binding. Furthermore, a concerted change of the first three (S219–F221) (spot 15) or first five (S219–P223) (spot 16) amino acids to alanine did not substantially decrease IgE binding (Figure 4). Thus the central IgE-binding region of this peptide is between E224 and V232. The most critical amino acids for IgE binding were identified




| Spot # | Peptide Sequence | Ala substitution |
|---|---|---|
| 1 | SGFAPEFLKEAFGV**A** | N233/A |
| 2 | SGFAPEFLKEAFG**A**N | V232/A |
| 3 | SGFAPEFLKEAF**A**VN | G231/A |
| 4 | SGFAPEFLKEA**A**GVN | F230/A |
| 5 | SGFAPEFLKE**A**FGVN | Wild type |
| 6 | SGFAPEFLK**A**AFGVN | E228/A |
| 7 | SGFAPEFL**A**EAFGVN | K227/A |
| 8 | SGFAPEF**A**KEAFGVN | L226/A |
| 9 | SGFAPE**A**LKEAFGVN | F225/A |
| 10 | SGFAP**A**FLKEAFGVN | E224/A |
| 11 | SGFA**A**EFLKEAFGVN | P223/A |
| 12 | S**A**FAPEFLKEAFGVN | G220/A |
| 13 | SG**A**APEFLKEAFGVN | F221/A |
| 14 | **A**GFAPEFLKEAFGVN | S219/A |
| 15 | **AAA**APEFLKEAFGVN | SGF/3A |
| 16 | **AAA**A**A**EFLKEAFGVN | SGFAP/5A |

**Figure 4. Alanine scanning of residues important for IgE binding in peptide 3 shown in Figure 3.** Fourteen peptides represented as spots 1–14 were manually synthesized on a SPOT membrane. Peptide 5 represented the wild-type sequence. Peptides 15 and 16 were synthesized with the first three or five amino acids substituted with alanine residues. Alanine substitutions are underlined and shown in bold. The membrane was probed with the soybean-sensitive sera (1:2 dilution) and bound IgE was detected with goat anti-human IgE–HRP (1:5000 dilution). The antigen–antibody complexes were detected by chemiluminescence.

as F225, L226, and F230. Other amino acids that contributed to IgE binding were G231, E224, and V232.

Comparison of the epitope in soybean G2a with corresponding peptides in soybean G1a and peanut Ara h 3. The new epitope on G2a identified here shares high sequence identity to the corresponding regions of soybean G1a and peanut Ara h 3. Out of the 15 amino acids in G2a, 6 and 5 are different in soybean G1a and peanut Ara h 3, respectively (Figure 5). Synthetic peptides based on the sequence of G1a and Ara h 3 corresponding to the equivalent region of the G2a epitope exhibited IgE binding (Figure 5). Based on the IgE-binding assay, the order of the IgE-binding ability to the synthetic peptides in the G2a epitope region was Ara h 3 (spot 6) ≥ G2a (spot 1) > G1a (spot 5) (Figure 5). Among the six amino acids shown to be crucial for IgE binding in

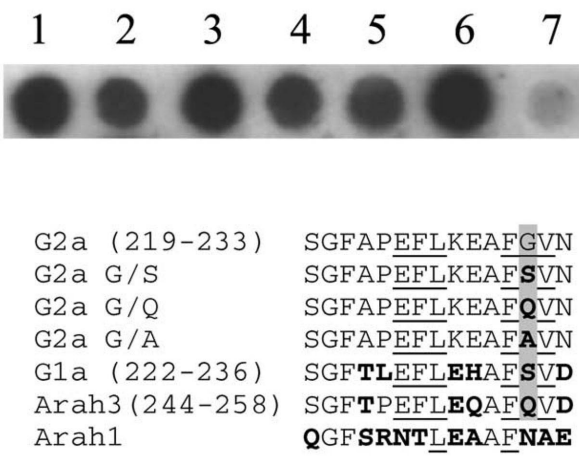



**Figure 5. Comparison of the new epitope from G2a with the peptides corresponding in sequence from soybean G1a and peanut Ara h 3.** Seven 15-mer peptides were manually synthesized on a SPOT membrane as spots 1–7. Spot 1 was the wild-type G2a epitope. Spots 2–4 were mutants, in which G231 of the G2a epitope was substituted with serine (peptide 2), glutamine (peptide 3), or alanine (peptide 4). Spots 5 and 6 correspond to the pertinent regions from soybean G1a and peanut Ara h 3, respectively. Spot 7 represented the corresponding region from peanut Ara h 1. The membrane was probed with the soybean-sensitive sera (1:2 dilution) and any bound IgE was detected using goat anti-human IgE–HRP (1:5000 dilution). The antigen–antibody complexes were detected by chemiluminescence.

G2a (see Figure 4), E224, F225, L226, F230, and V232 in G2a are conserved in G1a and Ara h 3. However, G231 in G2a corresponds to S234 in G1a and Q256 in Ara h 3 (Figure 5). Thus, we synthesized the G2a epitope peptide on a SPOT membrane with single amino acid substitutions, namely, G231 to serine or G231 to glutamine. The G231 to A231 mutant of the G2a epitope was used as a control. The strength of IgE binding to these peptides was Q231 (spot 3) ≥ G231 (spot 1) > S231 (spot 2) ≈ A231 (spot 4) (Figure 5). A peptide representing the corresponding region from peanut Ara h 1, which has 10 different amino acids as compared to the G2a epitope, did not show IgE binding (Figure 5).

Structural analysis of the identified G2a IgE epitope. The crystal structure for soybean glycinin G1 [22] was used to analyze the location of the G2a IgE epitope identified in this study. The tertiary structure of glycinin subunits is composed of a central jelly-roll β-barrel with α helical protrusions on either side [22]. The G2a IgE epitope encompasses an α helix at the subunit interface that is represented in red (Figure 6A). A surface map of the glycinin trimer reveals that this IgE epitope is accessible in the trimeric state (Figure 6B). Additionally, since trimers stack upon one another with their large surface area as the interface [22], this IgE epitope would also be accessible in the hexameric state.

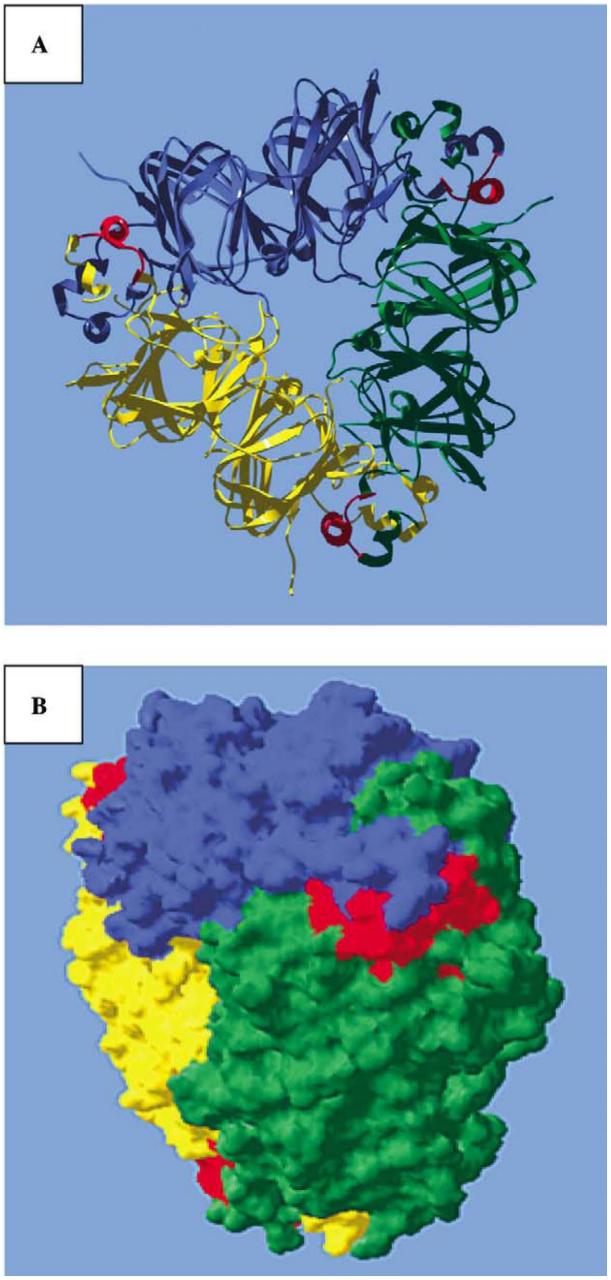



**Figure 6. Structural location of the G2a IgE epitope.** (A) The soybean glycinin G1 crystal structure [23] was used to model the location of the G2a IgE epitope. A ribbon diagram shows the individual subunits of the glycinin trimer represented in different colors. The G2a IgE epitope is represented in red for all three subunits. (B) A surface map of the glycinin trimer was generated with Swiss-PdbViewer and colored as in panel A. The trimer is rotated to show the surface-accessible area of the G2a IgE epitope.

## Discussion

In this study, we have focused on investigating conserved IgE epitopes of three well-documented allergenic glycinins, namely, soybean G1a and G2a and peanut Ara h 3. In previous studies, pooled sera from soybean-sensitive patients has been used to determine ep-

itopes of soybean G1a [17] and soybean G2 (including both acidic and basic chains) [18]. In comparing the epitope sequences reported in these studies, except for a seven amino acid overlap for one epitope, none of the other identified epitopes displayed significant sequence identity [17, 18]. This was somewhat surprising because soybean G1a and G2a proteins share significant amino acid identity in their acidic chains. However, the identification of different epitopes in these studies may be attributed to serum differences.

Our initial data revealed that the acidic chains of both soybean G1 and G2 recombinant proteins bind IgE (Figure 1B). We postulated that common epitopes can exist in these legume seed storage proteins and started our search with the stronger IgE-binding soybean protein, G2a. Using recombinant protein fragments of G2a, we established the dominant epitope region as residing on the C-terminal end of the G2a protein (Figure 2B). Subsequently the linear epitope was identified as the sequence S219–N233 (SGFAPEFLKEAFGVN) near the C-terminus of G2a (Figure 3). Another peptide E247–V261 showed weak IgE binding and was not examined further. Interestingly, this weak epitope has been previously identified in G2a [18], as well as in the corresponding sequences of G1a [17] and Ara h 3 [16]. Alanine scanning mutagenesis of the dominant linear epitope of G2a revealed that six amino acids (E224, F225, L226, F230, G231, V232) contributed most toward its IgE binding (Figure 4). The absence of the F230, G231, and V232 in peptide 2 and E224 and F225 in peptide 4 in Figure 3 may explain the lack of IgE binding to these peptides.

The dominant G2a epitope identified in this study is new as it was not among the five previously reported IgE-binding epitopes identified by Helm et al. [18] for soybean G2a. Interestingly, this G2a epitope was part of a corresponding region of a G1a epitope identified by our group in an earlier study [17] and was also adjacent to an Ara h 3 epitope (G240–A254) identified by Rabjohn et al. [16]. Structurally, this potentially allergenic epitope is located in a domain between two variable regions of legume glycinins, which have been identified as residing on the surface of the protein and susceptible to protease cleavage [12, 23]. In the tertiary structure of the glycinin trimer, this domain corresponds to the subunit interface region (Figure 6A). While the α helix encompassed by the G2a IgE epitope is not entirely surface accessible in the trimeric state (Figs. 6A and B), dissociation of the subunits would reveal the α helix as a protrusion that would be attractive for antibody binding. This portion of the glycinin molecule may represent a particularly allergenic domain of legume seed storage proteins, since several IgE-binding epitopes have been identified within this linear stretch of the glycinin and homologous vicilin sequences [16–18, 24, 25].

To test the hypothesis that this region represents an allergenic region of glycinins in general, we synthesized peptides based on the G2a epitope (Figure 5) that represented the corresponding region of G1a and Ara h 3. All of these synthetic peptides demonstrated IgE binding even though their apparent binding strengths were different. The synthetic peptide corresponding to the G1a sequence had less IgE-binding ability than the G2a epitope while the synthetic peptide corresponding to the Ara h 3 sequence displayed the strongest IgE binding among the three peptides. These results are consistent with the general observation that peanut seed proteins are more potent allergens than soybean proteins [3]. Out of the six amino acids in the G2a epitope important for IgE binding (Figure 4), only one amino acid, G231, was different in G1a (S234) or Ara h 3 (Q256). Interestingly, by substituting one crucial amino acid (G231/S or G231/Q) of the G2a epitope for the corresponding residue present in G1a or Ara h 3, we could mimic the IgE-binding ability observed for synthetic peptides that represented the sequences of these three glycinins (Figure 5). These results suggest that cross-reactivity for soybean- or peanut-allergenic individuals is a potential problem, especially since glycinins are the most abundant seed storage proteins. In summary, our current study has identified a common IgE-binding epitope site present in three related legume glycinin proteins: soybean G1a and G2a and peanut Ara h 3. This site could contribute to the cross-reactions observed in sera from peanut- and soybean-sensitive individuals.

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