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Cellular and Molecular Characterization of a Major Soybean Allergen

Abstract

Soybean proteins share a large number of cross-reacting allergens with other members of the legume family; however, soy-allergic patients rarely react clinically to other members of the legume family. Gly m Bd 30K, an IgE-binding protein with a molecular weight of 30 kD, was identified in soybean extracts by Western IgE-immunoblot analysis. This monomeric allergen was shown to have an N-terminal amino acid sequence and amino acid composition identical to that of the seed 34-kD protein, P34, a thiol protease of the papain family. Electron-microscopic immunolocalization of P34 monoclonal antibodies and IgE binding to sections of soybean seeds showed dense staining throughout the vacuolar bodies, localizing the allergens in protein storage vacuoles of seed cotyledons. We used pooled serum from soybean-sensitive patients to determine the linear IgE-specific epitopes in the 34-kD allergen amino acid sequence. B-cell epitope mapping revealed 10 regions of IgE-binding activity using an overlapping peptide strategy of 15-mers offset by 8 amino acids throughout the P34 sequence. Smaller overlapping peptides, 10-mers offset by 2 amino acids, revealed 16 distinct linear epitopes, 9 of which were mapped to the mature protein. No obvious amino acid sequence motifs could be identified by the smaller IgE-binding epitopes. Using individual patient serum, 5 immunodominant epitopes were identified in this allergen.

Key Words

Allergen
 Soybean
 Epitope
 Legumes
 Gly m 1
 Food hypersensitivity

Introduction

Approximately 8% of children and 1–2% of adults have some type of food allergy [1], with peanuts, milk, eggs, wheat and soybeans accounting for the majority of the allergic reactions [2]. Food allergies, due to the ingestion of seed proteins or seed protein products, have shown an increasing incidence in some countries, such as the USA and Japan, where peanuts and soybeans are responsible for many atopic disorders [3]. Because of their nutritional and functional benefits, soybean proteins are now being used increasingly in a number of food products [4]. New process-

ing methods have created a generation of soybean protein isolates with mild flavors and aromas, as well as improved functionality. These soybean proteins can be incorporated into a variety of food products at levels high enough to have an effect on the health of soybean-sensitive individuals. This has driven the food industry and soybean chemists to define the protein components responsible for soybean allergenicity, and to develop hypoallergenic soybean products.

Soybeans are a member of the legume family of plants that includes peanuts. Multiple protein allergens have been identified in soybean as well as in peanut extracts [5–7]. Al-

though cross-reacting proteins among soybeans and peanuts are immunochemically frequent, clinical responses to more than one are less common [8, 9]. These findings suggest that unique protein fractions in soybeans may be responsible for soybean allergic reactivity. A comparison of soybean IgE immunoblots of common legumes showed that soybean extracts shared several common as well as unique proteins with members of the legume family [10, 11]. Antigen-affinity chromatography for the removal of cross-reactive antibodies to soybean and peanut proteins has been used to identify 6 unique allergenic fractions of peanut and 2 unique allergenic fractions of soybean [12]. Experiments conducted to examine the stability of crude peanut and soybean allergens indicated that the antigenic determinants (IgE epitopes) of the major allergens in soybean and peanut were most probably linear epitopes [13].

An analysis of IgE-binding proteins in soybean extracts characterized by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE)/Western immunoblot analysis with sera from patients in Japan with atopic dermatitis revealed that about 20% of the patients tested had specific IgE antibodies to soybean proteins in the molecular weight range of 14–70 kD [14]. The individuals tested in this report did not experience severe or anaphylactic reactions to soybean proteins. In these investigations, IgE antibodies from 65.2% of the patients tested bound to a protein with a molecular weight of about 30 kD. This protein was given the allergenic identification of Gly m 1 [14]. The monomeric allergen was shown to have an N-terminal amino acid sequence and amino acid composition identical to that of the soybean 34-kD seed vacuolar protein, P34 [15–18]. The Genbank accession numbers, AB013289 and g170039, for Bd 30K (Gly m 1) and P34, respectively, show they are identical molecules (Genbank DataBase, 1998). The differences in the reported 4-kD molecular weight could be attributed to isolation methods (SDS-PAGE versus cDNA cloning), seed maturation, processing and expression following germination and cultivar isoform variation.

Our overall goal is to identify and characterize immunologically mediated adverse reactions to soybean proteins and specifically determine the presence of unique soybean protein epitopes in their amino acid structure. The prevalence of the hypersensitivity reactions to the allergen identified as Gly m 1, and its homology to the previously identified protein, P34, a constituent of protein storage vacuoles in soybean seed cotyledons, made this protein an ideal candidate for identification and characterization of the major IgE-binding epitopes. The specific aim of the present investigation was to identify any continuous IgE-binding epitopes within the amino acid sequence of P34 in order to begin to

understand the molecular and immunochemical characteristics of legume proteins that predispose them to be allergens and that may contribute to their unique hypersensitivity response in sensitive individuals. In this communication, we report the localization of the soybean allergens to the protein storage vacuoles in the seed cotyledon and the identification of 5 distinct IgE-binding epitopes that appear to be immunodominant epitopes in this major soybean allergen.

Materials and Methods

Serum IgE

Four patients with atopic dermatitis and a positive immediate skin test response to soybean extracts were confirmed to be sensitive to soybean protein by double-blind, placebo-controlled food challenge [9]. Equal volumes of sera from these 4 individuals were combined to prepare a serum pool containing soybean-specific IgE antibody. A plasma pool from 7 individuals reporting soybean allergy and containing soybean-specific IgE antibody, as determined by SDS-PAGE/Western IgE immunoblot analysis, was prepared from plasma purchased from PlasmaLab International (Everett, Wash., USA). The serum pools or individual sera were diluted 1:20 (v/v) in Tris-buffered saline (25 mM Tris-HCl, pH 7.2, 150 mM NaCl) with 0.05% Tween 20 (TBST) for immunoblot analysis and in 1:5 (v/v) TBST with blocking solution, as recommended by the manufacturer (Genosys Biotechnologies Inc., The Woodlands, Tex., USA), for the mapping of IgE-binding regions and epitopes of the Gly m 1 allergen.

Soybean Extracts

Preparations of defatted soybean flakes were obtained from commercial sources (Dr. Jim Brooks, Ross Laboratories, Columbus, Ohio, USA). Defatted (Soxhlet extraction with petroleum ether) soybean flakes were ground to a fine powder in a mortar and pestle and extracted (1:20 w/v) in 20 mmol sodium phosphate, 1 mmol NaCl, pH 7.2, overnight at 4°C.

Electron Microscopy and Immunochemistry

Soybean seed cotyledons were cut approximately 1 mm³ and fixed with 4% formaldehyde and 2% glutaraldehyde in 0.1 M potassium phosphate buffer, pH 7.4, overnight at 7°C. The tissue pieces were dehydrated in a graded ethanol series and embedded in the hard formulation of LR White resin. Ultrathin sections mounted on nickel grids were blocked with 10% fetal bovine serum in TBST for 15 min at room temperature. The sections were then incubated for 60 min at room temperature with either serum from peanut-hypersensitive individuals or a control serum (1:10 v/v) from an individual with no known peanut sensitivity. The grids were then washed with TBST and incubated with goat anti-IgE (KPL, Gaithersburg, Md., USA) (0.1 mg/ml in 10% fetal bovine serum). The grids were again washed and indirectly labeled with 10 nm colloidal gold particles coupled to rabbit anti-goat IgG (Ted Pella Inc., Redding, Calif., USA). The grids were washed with TBST, rinsed in water and stained with 5% uranyl acetate. Then they were examined and photographed with a Philips 400T electron microscope.

SDS-PAGE/Western Immunoblot Analysis

SDS-PAGE was performed according to the method of Laemmli [19]. All gels were composed of 12.5% acrylamide-resolving gel and 4% acrylamide-stacking gel. Electrophoretic transfer of proteins and immunoblotting was performed according to standard procedures [20]. The blots were blocked with TBST 60 min prior to incubation with antibodies diluted in TBS buffer for at least 12 h at 4°C or for 4 h at room temperature. Primary IgE antibody was detected with radiolabeled anti-IgE antibody (Sanofi Pasteur Institute, Chaska, Minn., USA) diluted 1:5 (v/v) in TBS buffer. Blots were washed 3× in buffer, air dried and exposed to Kodak X-Omat x-ray film at -70°C for 18–72 h and developed in an automatic developer (Konica SRX-101). Mouse monoclonal IgG antibodies to P34 were diluted 1:20 v/v in TBST buffer and detected using the ECL Western blotting procedure (Amersham Life Sciences Inc., Arlington Heights, Ill., USA). Primary antibody was incubated with the blot for 1 h at room temperature, washed 3×, incubated with HRP-labeled secondary antibody (1:50,000 v/v) for 1 h at room temperature, washed 3× and detected with Hyperfilm ECL, as recommended by the manufacturer.

B Cell Epitope Mapping

The predicted amino acid sequence of P34 [15] was used to generate individual peptides for IgE-binding studies. Individual peptides were synthesized on a cellulose membrane containing free hydroxyl groups, using Fmoc amino acids according to the manufacturer's instructions (Genosys Biotechnologies, The Woodlands, Tex., USA). Synthesis of each peptide was started by esterification of an Fmoc-amino acid to the cellulose membrane. After washing, all residual amino functions on the sheet were blocked by acetylation to render the cellulose membrane unreactive during the subsequent steps. Each additional Fmoc amino acid was esterified to the previous one by this same process. After addition of the last amino acid in the peptide chain, the amino acid side chains were deprotected using a mixture of dichloromethane/trifluoroacetic acid/triisobutylsilane (1/1/0.05, v/v), followed by treatment with dichloromethane and washing with methanol. Membranes containing synthesized peptides were washed with TBS and then incubated overnight at room temperature with the Genosys blocking solution. The membranes were either probed immediately with plasma or serum IgE as described above, or stored at -20°C until used. IgE binding was determined by incubating cellulose membranes containing the synthesized peptides with serum from individuals with documented soybean hypersensitivity and, as control, individual serum from an individual with high-titer IgE with no known food allergy. Primary antibody was detected with radiolabeled anti-IgE as described for the autoradiographs of the SDS-PAGE/Western IgE immunoblot analysis.

Results

Localization of Soybean Allergens to Protein Storage Vesicles

Many of the proteins that have been identified as allergenic in legumes are seed storage proteins. In an effort to determine where the IgE-binding proteins of soybean cotyledons were localized, electron microscopic immunolocalization studies using clinically relevant soy-

bean-specific serum and mouse monoclonal antibody to P34 were performed. Thin sections of soybean cotyledons incubated with IgE antibody (fig. 1A) or with the mouse monoclonal antibody specific to P34 protein (fig. 1C) revealed dense staining in the protein storage vacuoles with less dense staining in the remaining cell organelles. No dense labeling was identified using a human control serum with a high IgE titer to nonsoy proteins (data not shown) or by omission of the primary mouse monoclonal antibody (fig. 1B).

Identification of Soybean Allergens in Soybean Extracts

SDS-PAGE analysis and immunolocalization of proteins in crude soybean extracts by immunoblot analysis using serum IgE from soybean-sensitive individuals and the monoclonal antibody to P34 are shown in figure 2. Multiple Coomassie blue staining bands ranging from <20 to >65 kD can be identified in figure 2 (lane 2). IgE specific binding proteins are shown in figure 2B (lane 2), using serum from the individuals clinically sensitive to soybean. Prominent IgE-binding bands can be seen at 20, 30–35, 50 and 65 kD. No IgE binding could be detected with the control serum (data not shown). The results following the localization of the P34 antigen with monoclonal antibody are evident as a prominent 30-kD protein, corresponding to one of the IgE-binding proteins shown in figure 2C (lane 2). The higher-molecular-weight staining material is believed to be due to the aggregation of the P34 antigen or to the association of the P34 protein with other molecules in the extract that were not completely dissociated under our conditions. We did not detect staining with control monoclonal antibodies (data not shown).

Multiple Linear IgE-Binding Epitopes Exist on the P34/Gly m 1 Protein

Since the P34/Gly m 1 protein was shown to be a major IgE-binding protein [14], we set out to identify the IgE-binding epitopes within this allergen. To accomplish this, we synthesized 61 overlapping peptides representing the entire length of the protein to determine the IgE-binding regions recognized by individuals clinically sensitive to soybean. Each peptide was 15 amino acids long and offset from the previous peptide by 8 amino acids. In this manner, the entire length of the protein could be studied in large overlapping fragments. These synthesized peptides were probed with either a 4-patient serum pool with documented soybean hypersensitivity or a control serum from an individual with a high IgE titer with no known food allergy. Twenty-four of the 61 peptides localized in the ten regions designated D1–10 were identified that bound IgE (fig. 3). Serum

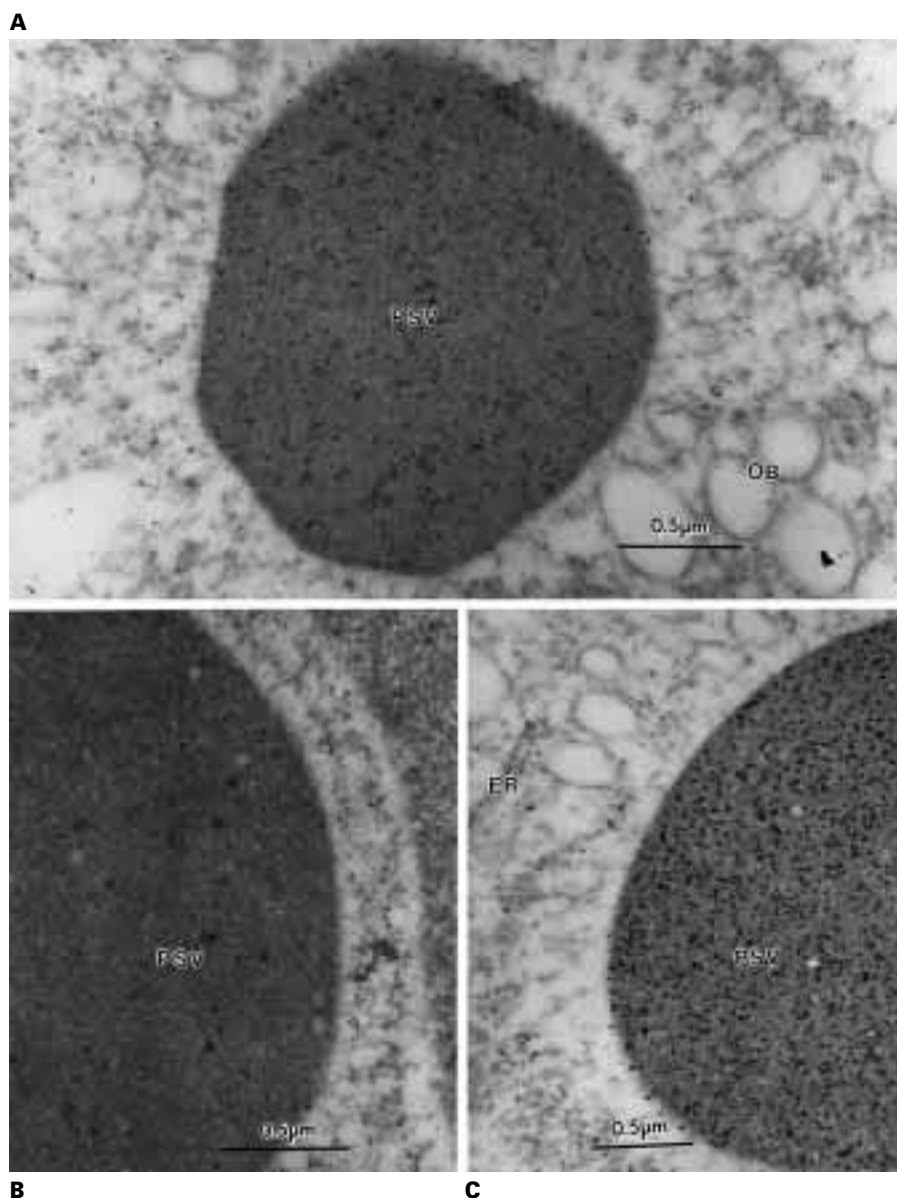


Fig. 1. Immunocytochemical analysis of soybean cotyledons using human serum IgE and murine monoclonal antibodies to a P34 protein. **A** Pooled serum from 5 clinically soy-sensitive individuals. **B** Negative control; no primary antibody. **C** Murine monoclonal antibody prepared against P34 protein. PSV = Protein storage vesicle; OB = oil body; ER = endoplasmic reticulum.

from the control individual did not recognize any of the synthesized peptides (data not shown).

To determine the minimal amino acid sequence required for IgE binding in these ten regions, smaller overlapping peptides (10 amino acids overlapped by 8) spanning each region (D1–10) were synthesized. This made it possible to determine individual IgE-binding epitopes within the larger IgE-binding regions of the P34 protein. First, using our serum pool, we were able to identify the 16 epitopes identified in figure 4B. In an effort to determine which, if any, of the 16 peptides could be identified as immunodominant (reacting with >50% of the serum IgE of tested patients), individual sets of the 16 peptides were probed with serum

from our 4 individuals clinically sensitive to soybean. A representative peptide blot using serum from a single individual is shown in figure 4A. The IgE in these individuals' serum recognized peptides 1, 3, 5, 6, 8–13, 15 and 16. The remaining 3 individual sera were tested in the same manner and the intensity of IgE binding to each spot was densitometrically scanned to determine the relative intensity of IgE binding of the 16 peptides (fig. 5). IgE from all patients recognized multiple peptides. The most common amino acids comprising $\approx 30\%$ of all amino acids represented in the peptides binding IgE were 2 acidic amino acids (aspartic acid and glutamic acid) and 3 basic amino acids (histidine, lysine, and arginine). There was no obvious amino acid se-

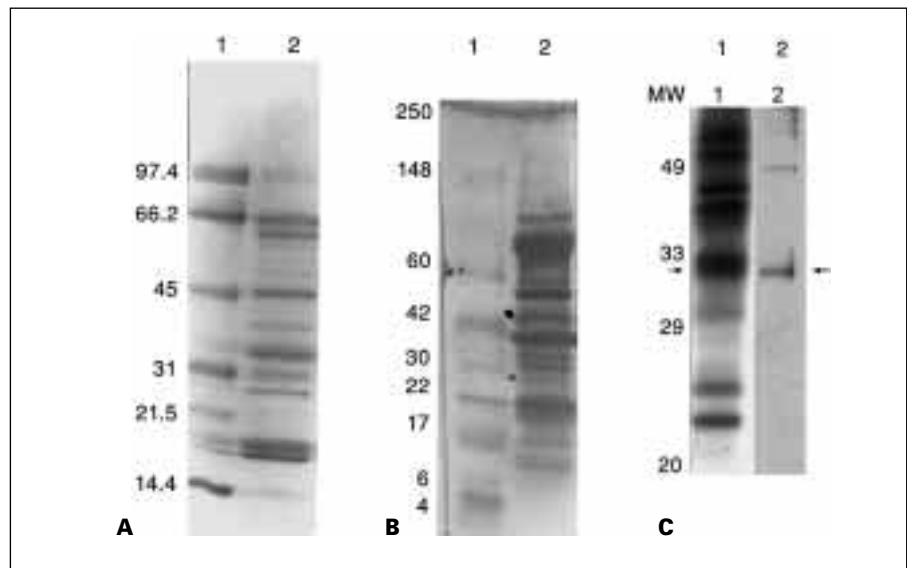


Fig. 2. **A** Coomassie-blue-stained SDS-PAGE/Western blot of crude soybean protein extract. Lane 1 = molecular weight (MW) size of prestained markers; lane 2 = amido-black-stained nitrocellulose blot of crude soybean extract. **B** Immunoblot analysis of soybean proteins with serum IgE from soybean-sensitive individuals. Lane 1 = molecular weight size of prestained markers; lane 2 = autoradiograph of a Western immunoblot with soy-specific IgE antibody using radio-labeled secondary antibody (Sanofi Pasteur Diagnostics). **C** Identification of the soybean allergen, P34/Gly m 1 using serum IgE from

soybean-sensitive individuals and monoclonal antibody to P34. Molecular weight is indicated to the left of the panel. Lane 1 = Immunoblot analysis of soybean proteins with serum IgE from soybean-sensitive individuals; lane 2 = chemiluminescence of a Western immunoblot with P3E1 P34 monoclonal followed by HRP-labeled secondary antibody and detected by Hyperfilm ECL using the ECL Western blotting procedure (Amersham). The arrows indicate the position of P34/Gly m 1.

quence motif shared by all the IgE-binding epitopes. On the basis of IgE binding (> 10%) and prevalence (serum from 2 or more individuals), epitopes 1, 6, 13, 15 and 16 appear to be the immunodominant epitopes on this allergen.

Comparison of Soybean Allergen P34/Gly m 1 Epitope Amino Acid Sequence to Der p 1 Thiol Proteases

Since P34 [16] and the mite aeroallergen Der p 1 [21] have been identified as cysteine/thiol proteases (members of the papain superfamily of proteins), we compared the amino acid sequence of P34 to Der p 1 thiol protease using gene bank sequence data. Figure 6 shows the complete alignment of the sequences of these proteins. Amino acid comparison of the P34/Gly m 1 sequence showed a 54% similarity and 30% identity when compared to Der p 1. In the 6 epitopes representing the sequence homology between Der p 1 and P34, only 1–4 amino acids/epitopes were identical between the shared sequences. The relationship for shared allergenicity will require additional determinations to establish the relevance of potential cross-reactivity.

Discussion

Because antigen-specific IgE plays such a critical role in cellular interactions in allergic disease, determination of allergen-specific, IgE-binding epitopes is an important first step toward the understanding of this complex disease process. In the present study, we have determined by electron-microscopic immunochemical localization that the majority of allergenic proteins in soybean cotyledons are contained in the seed protein storage vesicles. Furthermore, we have documented that there are multiple linear IgE-binding sites on the P34/Gly m 1 allergen.

Various studies have identified multiple allergens in soybean extracts which may cross-react immunochemically with proteins in other members of the legume family [4, 8]. However, another study [23] showed that 87% of patients tested were skin prick test positive for peanut, 43% were skin prick tested for soybean with a positive food challenge of 45 and 14.5%, respectively. This study suggests that clinical symptoms for more than one legume are rare in children. This is somewhat surprising, since soybean and peanut belong to the same family, Leguminosae, and the

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1      10      20      30      40      50      60      70      80
MGFLVLLLFSLGLGSSSSSISTHRSILDLDTCKPTTOKQVSSLPQLNKSEHGGRVYHNSEEEAKPLEIFKNNNSNYLRDYNANRY
D1      D2      D3
Peptides 1-2      Peptides 4-5-6-7      Peptides 10-11
MGFLVLLLFSLGLG      SISTRHSILDLDTCK      YHNSEEEAKPLEIFK
      LDFSLGLGSSSSSIS      KILDLDTCKPTTQKQ      EAKRLEIFKNNNSNYI
      LCKPTTOKQVSSLPQ
      QKQVSSLPQLNKSEH

      90      100      110      120      130      140      150      160
SIRSHRLGLNKFADITPQEPFKAYLQAPKDVSSQQLKMANXKMKKEQVSCDHPFASNDWRKKQVITQVKYGGCGRGNAFSATG
D4      D5
Peptides 17-18-19-20      Peptides 23-24
DITPQEPFKAYLQAP      CHFPASNDWRKKQVI
      FSKKYLQAPKDVSSQ      WDWRKKQVITQVKYQ
      QAPKDVSSQQLKMANX
      HQQLKMANXKMKKEQ

      170      180      190      200      210      220      230      240
AIEPAHAIAAGDLVELSEQELVDQVESSSSCYNGKHYSQSFENVLEHGOIATDDDYFYRAKQGRCKANKIQDKVTIDG
D6      D7
Peptides 33-34      Peptides 36-37-38
RSEKRYNGWHYQSPF      EHGGIATDDDYFYRA
      NGWHYQSFENVLEHG      THDDYFYRAKQGRCK
      YRAKQGRCKANKIQD

      250      260      270      280      290      300      310      320
YETVIMSDSESTESSETEQAFLSAILEQPIESVSTAKDFHLYTGGIYDGENCTSPYGINHFFVLLVGYGSAAGVDYNIAXNSNG
D8      D9
Peptide 40      Peptides 50-51
ISVSTAKDFHLYTG      SPYGINHFFVLLVG
      HFVLLVGYGSAAGVD

      330      340      350      360      370
EEWCELDGYIWIQRNTGNLLQYCGMNYFASYPTKKESETTLVSARVKGHRVDHSPL
D10
Peptides 55-56
EEWCELDGYIWIQRNT
      GYIWIQRNTGNLLGV

↓ = MATURE PROTEIN

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Fig. 3. P34/Gly m 1 amino acid sequence with IgE-binding 15-mers overlapped by 8 amino acids. The entire amino acid sequence of the P34 protein, GenBank No. g170039 was synthesized into 15 amino acid sequences overlapped by 8 amino acids. The 61 peptides representing the entire sequence were then incubated with serum from soybean-sensitive individuals and bound IgE identified by radiolabeled anti-IgE and autoradiography. Twenty-four peptides bound IgE and were localized in the ten regions, identified as D1–D10 in this initial screening.

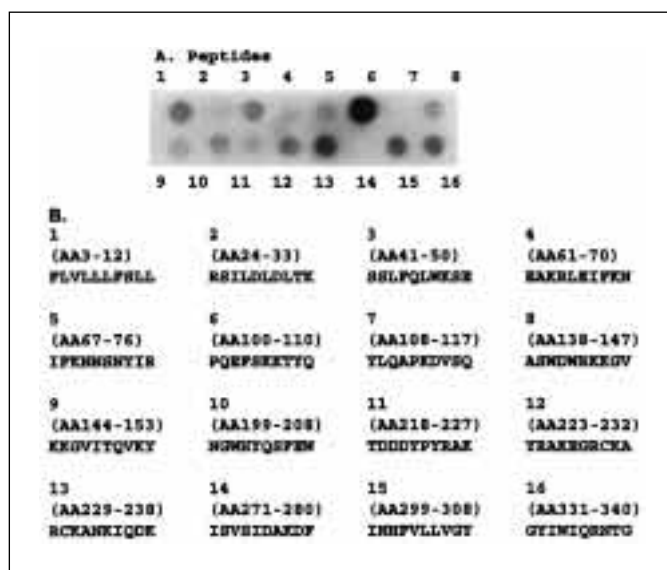


Fig. 4. A A representative SPOTS (Genosys) membrane autoradiograph of the 16 IgE-binding epitopes from P34/Gly m 1 with serum from a single soybean-sensitive individual. Each spot (left to right) represents a peptide previously identified as an IgE-binding epitope using pooled serum from soybean-sensitive individuals. **B** Amino acid sequence and position of the 16 epitopes in the linear sequence of P34/Gly m 1.

same subfamily, Papilionoideae. This also suggests that there must be unique IgE-binding proteins present in both peanut and soybean extracts that are responsible for the diverse clinical reactions identified for these two legumes. Indeed, several unique antigenic fractions of peanut and soybean extracts have been identified in IgE-antibody-binding studies with sera in which either soybean or peanut cross-reacting antibodies were removed by affinity chromatography [12]. On the basis of this information, we hypothesized that soybean allergens must possess unique IgE-binding epitopes that will enable the preparation of more accurate and specific reagents for in vitro and in vivo testing.

Overall, reports indicate that the P34 protein represents approximately 2–3% of the total seed protein [15], while the glycinins and β -conglycinins (the other major soybean allergens) represent 70–80% of the total seed protein [24], depending on the soybean seed variety. To our knowledge, a thiol protease homologous to P34/Gly m 1 has not been identified in peanut cotyledons. However, we would suggest that the clinically relevant epitopes in these two legumes are different. This may help to explain some of the differences in the clinical reactivity, following the ingestion of these two legumes. As suggested by the number of

shared amino acids between Der p 1 and P34 in what we identified as soybean epitopes, there are as yet unidentified factors that contribute to the respective allergenicity of a family of proteins, such as cysteine proteases. More detailed comparisons of allergenic structure and epitope mapping with allergen-specific IgE from clinically sensitive patients will be required to distinguish these factors.

The documentation of multiple linear IgE-binding epitopes distributed throughout the entire length of the P34/Gly m 1 soybean protein is not novel. In our investigations with peanut allergen Ara h 1 and Ara h 2 [25, 26] and work derived from other allergen studies including cow's milk [27], codfish [28], hazel [29] and shrimp [30], all have been shown to contain multiple IgE-binding epitopes. It is noteworthy that peptides from Der p 1 that released histamine from peripheral blood [22] did not have any amino acid sequences in common with IgE-binding epitopes from soybean-sensitive individuals. A linear amino acid sequence comparison of soybean IgE-binding epitopes of P34/Gly m 1 protein with the respective homologous linear amino acid sequences identified for the Der p 1 thiol protease allergen showed a similar use of D, R, T, Y, C and G amino acids. The essential importance of these 6 amino acids for IgE binding in the respective soybean and mite allergens will need further investigation. These observations likely reflect the polyclonal nature of the normal human IgE response.

Another advantage of characterizing the IgE-binding epitopes would be the potential immunotherapeutic treatment of patients suffering from soybean hypersensitivity. Unlike patients with peanut hypersensitivity, who do not lose their allergic reaction to peanuts throughout their lifetime, patients with soybean hypersensitivity tend to become tolerant of soybeans in 3–5 years following diagnosis. The development of immunotherapeutic strategies to enhance or shorten this process of tolerance would prevent several years of tedious dietary avoidance of soybean proteins in prepared foods. The identification of soybean-specific IgE-binding epitopes from clinically sensitive individuals will allow the preparation of purified or recombinant allergenic or nonallergenic peptides that may prove beneficial as both immunotherapeutic and diagnostic agents.

Future studies with these epitopes, and epitopes identified on other soybean allergens, should allow the identification of amino acids within these epitopes that can be mutated to decrease specific IgE-binding to soybean allergens. Once identified, the soybean allergen genes could be altered and replaced with nonallergenic homologs in the soybean genome. The development of less allergenic soybean plants may prevent the initial sensitization of individuals geneti-

Fig. 5. Identification of the immunodominant P34/Gly m 1 epitopes. IgE-binding peptides (1–10) recognized from the pooled soybean-sensitive sera were synthesized as 10-amino-acid peptides and probed with serum from 4 individuals with documented soybean hypersensitivity. IgE-binding to the peptides was detected by radiolabeled anti-human IgE. The relative intensity of IgE binding to each peptide was determined by densitometry and expressed as a percentage of the individual's IgE binding to the peptides. Each panel represents the peptide IgE-binding pattern obtained from an individual serum donor.

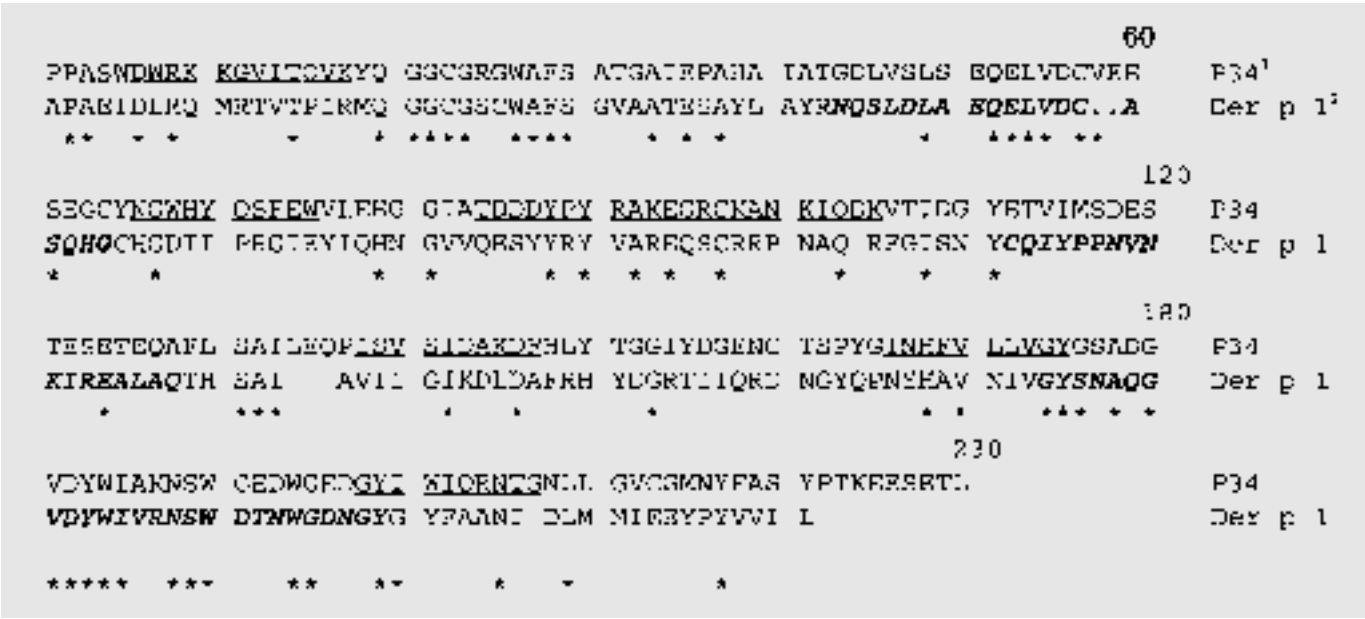
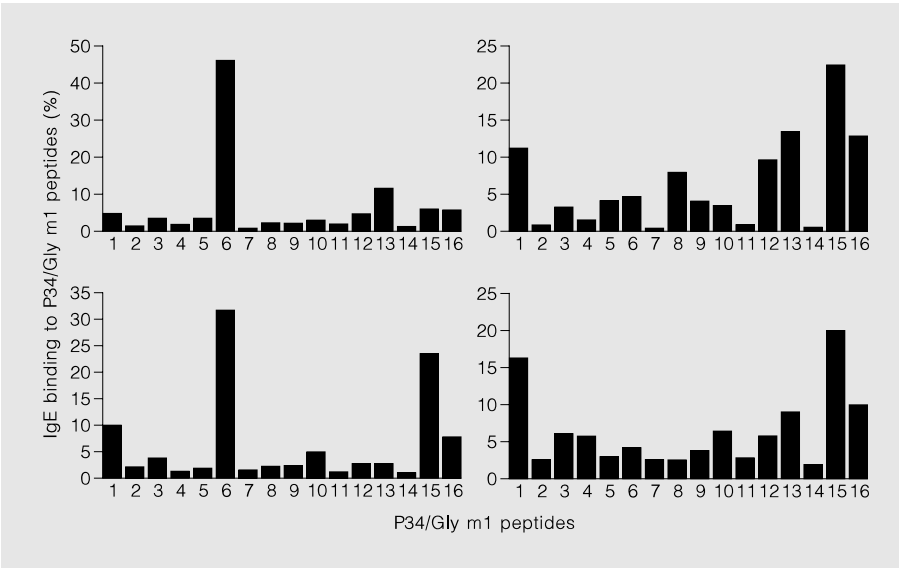


Fig. 6. Sequence alignment of the P34/Gly m 1 and Der p 1 thiol proteases. The amino acid sequences for each protein were obtained from the GenBank database and aligned according to sequence homology using the GenBank system. * = Identity of amino acids in the peptide sequences. Underlined amino acids identify the soybean epitopes recognized by the serum IgE of clinically relevant soybean allergic individuals. Bold, italicized amino acids identify the Der p 1 peptides that released histamine [30]. ¹Amino acid sequence for soybean protein P34, GenBank No. g170039. ²Amino acid sequence for *Dermatophagoides pteronyssinus* (Der p 1), GenBank No. U11695

cally predisposed to the development of food allergy. Obviously, a prudent approach would require that the altered soybean gene retains as much of its native function and properties as possible. With the widespread use of soybeans in consumer foods and the prevalence of soybean hypersen-

sitivity reactions, this possibility is actively being pursued in our laboratories.

Collectively, these studies clearly indicate the importance in defining clinically relevant IgE-binding soybean epitopes that will improve their diagnostic value. The iden-

tification and characterization of the major allergens of soybeans and their immunodominant epitopes should have a significant impact on the diagnosis of soybean allergy and in our understanding of the mechanisms of IgE-mediated reactions. The elucidation of unique soybean proteins and/or epitopes will enable a more accurate prediction by either in vivo or in vitro testing.

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