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Immunochemical reactivity of soybean β-conglycinin subunits

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Abstract
The immunochemical reactivity of two polyclonal sera raised against soybean purified αα′ and β subunits was analysed. We show that anti-αα′ serum mainly reacts with αα′ subunits and β′ subunit, but it does not recognize β subunit. In addition, β subunit is not able to compete with αα′ subunit for anti-αα′ serum recognition sites; therefore the main immunogenic region of αα′ subunits is the extension region. Both anti-αα′ and anti-β sera were able to recognize *E. coli* produced α subunit, consequently glycosylation is apparently not required for antigen–antibody interaction. Anti-β subunit serum shows strong reactivity against soybean produced β-subunit and recombinant α subunit, while it shows weak recognition of soybean purified αα′ subunits. A mutant of α subunit with the N terminal β barrel region and C terminal α helices deleted, failed to be recognized by anti-β serum. For that reason although N and C terminal domains are structurally equivalent, their immunochemical reactivity is different.

Keywords: *β-conglycinin, recombinant soybean subunits, immunoreactivity, soybean 7S globulin.*

Introduction

Soybean proteins are used as a supplement for the preparation of many food products since they have good nutritional and physicochemical properties (such as emulsifying and gel forming abilities) (Utsumi 1992; Utsumi et al. 1997). β-Conglycinin and glycinin, the major soybean proteins, are the main reason for these properties (Utsumi 1992; Utsumi et al. 1997). β-Conglycinin (7S globulin) is a trimeric protein composed of three major subunits: α, α′, and β, (Thanh & Shibasaki 1976, 1978). Their names derive from the first immunological studies showing that α and α′ share antigenic determinants, while the β subunit is immunologically different (Thanh & Shibasaki 1977). Other minor subunits are also present in this 7S fraction and are named γ and δ subunits (Thanh & Shibasaki 1977) and β′ subunit (Coates et al.

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1985). Minor β-conglycinin subunits have not being further studied while major subunits have been cloned and their sequences determined (Doyle et al. 1986; Harada et al. 1989; Sebastiani et al. 1990). Alignment of the amino-acid sequences of α, α′, and β subunits indicates that they have in common a region named core region; and that α and α′ subunits contain an additional 150 amino acid sequence, named extension region, at the N terminus which is rich in acidic amino acids residues (Doyle et al. 1986; Harada et al. 1989; Sebastiani et al. 1990; Maruyama et al. 1998). The core region (420–440 residues) among α, α′ and β subunits shows high homology degree (90.45, between α and α′, 76.2% between α and β, and 75.5% between α′ and β) (Maruyama et al. 1998). However, it is known that the contributions of individual subunits to physicochemical and physiological properties and allergenicity are different (Ogawa et al. 1995; Lovati et al. 1998; Maruyama et al. 1998).

Immunochemical properties of β-conglycinin subunits have scarcely been studied. Reported studies based on the use of antibodies raised against the whole β-conglycinin were used to identify constituent subunits of soybean 7S fraction (Thanh & Shibasaki 1977; Yamauchi et al. 1981; Iwabuchi & Shibasaki 1981; Iwabuchi & Yamauchi 1984; Iwabuchi & Yamauchi 1987) and to follow structural changes during thermal treatment of soybean proteins (Plumb et al. 1994, 1995; Huang et al. 1998). By using monoclonal antibodies raised against the whole β-conglycinin, the extension region of α and α′ subunits was identified as the main immunogenic region (Plumb et al. 1995). Recently soybean protein immunoassays have acquired importance because their involvement in food allergy (Brandon and Friedman 2002). In this context, β-conglycinin is the third main allergen responsible for soybean allergy, but only the α subunit is identified as having reactivity with soybean-sensitive-patients sera (Ogawa et al. 1995).

Taking into account the high similarity in the core region of β-conglycinin subunits and the differences in their immunogenic and allergenic properties described above, we were interested in studying the immunochemical properties of individual β-conglycinin subunits and characterizing their main immunogenic regions. In this work, two polyclonal sera, one against αα′ subunits and the other against β subunit, were produced and their reactivity with soybean isolated and recombinant subunits was analysed.

Materials and methods

Preparation of soybean protein isolated and 7S fraction

Soybean protein isolated was prepared as described previously (Petruccelli & Añón 1994). Crude soybean 7S fraction was obtained as described by Iwabuchi and Yamauchi (1987).

Purification of β-conglycinin subunits by anion exchange chromatography

α; α′ and β subunits were separated using Q-Sepharose chromatography under dissociating conditions with the help of a FPLC system (Pharmacia, Sweden) essentially as described by Coates et al. (1985). Briefly, the sample (soybean isolate or 7S crude fraction) was dissolved in buffer A (20 mM sodium phosphate; 6 M urea; pH 7.5) and was loaded into the column previously equilibrated with buffer A plus

10% NaCl. Bound proteins were eluted with NaCl gradient: β′ subunit and β subunit were released at 12.5% and 15% NaCl; while α′ and α subunits were released at approximately 30% NaCl.

Production of mice polyclonal antibodies

Two eight-week-old BALB/c mice groups (four mice per group) were immunized by intraperitoneal injections with 50 μg of either αα′ subunits or β-subunit in 100 μL of PBS (140 mM NaCl; 2.7 mM KCl; 1.5 mM KH_2PO_4; 8.1 mM Na_2HPO_4; pH 7.4) mixed with the same volume of Freund's complete adjuvant (Sigma Chemicals, St Louis, MO). Booster injections were similarly administered every two or three weeks during a period of three months, with complete replacement with incomplete adjuvant. Mice were bled a week after the last immunization.

cDNA library construction and cloning

Total RNA was extracted from developing soybean (*Glycine max* L. Merr.) seeds and a cDNA library was constructed using the Lambda Zap cDNA Library Construction Kit (Stratagene, La Jolla, CA). The Full-Length cDNA coding sequence of α subunit of β-conglycinin (Gen-Bank Accession Number X17698) was obtained by PCR amplification with the oligonucleotides primers 5′α (CTAG*TCTAGA*GTATGATGAGAGCACGGTTCCC, *Xba I* site in italics) and 3′α (CGC*GGATCC*TCAGTAAAAAGCCCTCAAAATTG, *Bam HI* site in italics). The coding sequence for α COOH terminal domain (α-CTD) was also obtained by PCR amplification with the primers 5′α-CT (CTAG*TCTAGACATATG*TAAACACTCGCATACCCGTT, *Xba*/*NdeI* site in italics) and 3′α-CT (CGCG*GGATCC*TCACATTGTCTTTCGAACCTGCAAG, *BamH1* site in italics). The last primers allow the amplification of the region between 920–1648 nucleotides from the start codon of α subunit that encoding to amino acids 305–539 of this subunit. The PCR products were digested with *Xba I* and *Bam HI* and inserted into the plasmid pBluescript SK (Stratagene) restricted by the same enzymes. The resulting constructs, named pSK-α and pSK-αCTD, were confirmed by sequencing.

Constructs for expression in Escherichia coli

To construct the plasmid for expression in *Escherichia coli*, the α-CTD coding sequence was released from pSK-αCTD with *Nde I* and *Bam HI* and cloned into the expression vector pET28a (Novagen, San Diego, CA) restricted with the same enzymes. In the case of α subunit, the coding sequence for the mature protein was amplified by PCR with the primers 5′α-62 (CTAGCAT*CATATG*GTGGAGAAAGAAGAATGTGAAGAA, *Nde I* site in italics) and 3′α. The first primer was designed to remove 62 amino acids from the N terminal domain including 25 amino acids sequence of the signal peptide and 37 amino acids propeptide sequence (Doyle et al. 1986). The obtained PCR product was also introduced into pET28a. These plasmids were named pEt-αCTD and pEt-mα, respectively.

Production of soybean 7S subunits in E. coli *and purification*

pEt-αCTD and pEt-mα were transformed into *E. coli* BL21 (Novagen, San Diego, CA). Cells were grown at 37°C until absorbance at 600 nm reached 0.5–0.6, and then were induced with 0.5 mM isopropyl b-D-thiogalactopyranoside for 6 h at 37°C. The cell pellets were resuspended in lysis buffer (20 mM Tris-HCl; 500 mM NaCl; 0.1% (v/v) Nonidet P-40; 1 mM phenylmethylsulfonyl fluoride; 1 mg mL^{-1} lysozyme; 10 μg mL^{-1} DNAase and 10 μg mL^{-1} RNase; pH 7.5). Lysis was completed using a sonicator. Under these conditions, proteins were accumulated in inclusion bodies. The pellet was washed three times with the lysis buffer, then inclusion bodies were dissolved in 50 mM Tris-HCl; 500 mM NaCl; 6 M urea; pH 7.5. Afterward proteins were bound to a nickel resin (Novagen); unbound proteins were eliminated by washes with 10 mM Tris-HCl; 0.1 M NaH_2PO_4; 6 M urea; pH 6.3. Finally, His-Tag proteins were eluted with 10 mM Tris-HCl; 0.1 M NaH_2PO_4; 6 M urea; pH 4.5. The proteins were dialyzed to eliminate the urea and then stored at −80°C.

SDS-PAGE and immunoblot analysis

Samples were subjected to SDS-PAGE followed by either Coomassie blue staining or blotting to nitrocellulose membranes (Schleicher & Schuell Bioscience, Inc., USA). The membranes were treated with anti-αα′ or anti-β sera followed by incubation with HRP conjugated anti-mouse secondary antibody (BioRad Laboratories, CA). Immunoreactive signals were detected with either 4-cloro-1 naphthol or chemiluminescent substrate (SuperSignal West, Pierce, Rockford, IL) and exposure to X-ray film.

Sequential competitive ELISA

Assay was optimized according to the method described by Chirdo et al. (1995). Briefly, plastic wells (Maxisorp, Nunc, Denmark) were coated with either αα′ or β subunits by passive adsorption for 16 h at 4°C. After that, the plates were blocked with 3% skimmed milk. Competition was carried out by preincubating various concentrations of either αα′ or β subunits with anti-αα′ or anti-β sera for 2 h at 37°C. Subsequently, these immunocomplexes were added to the plates, and incubated for 30 min at 37°C. Afterwards, the plates were incubated with HRP conjugated anti-mouse secondary antibody for 1 h at 37°C. Finally the color reaction was developed by adding a solution containing o-phenylenediamine (1 mg mL^{-1}, Merck, Germany) and 30% hydrogen peroxide (1 μl mL^{-1}) in 0.1 M citrate-phosphate buffer, pH 5. The enzymatic reaction was stopped after approximately 20 min with 40 μL/well of 2 M sulfuric acid. The absorbance was measured at 420 nm.

Results

Characterization of mice polyclonal antibody

β-Conglycinin subunits were purified with the purpose of preparing polyclonal sera. To this end, β-conglycinin crude extract was dissociated with urea and its subunits were separated using anion exchange chromatography mainly in two fractions: one containing the β subunit and the second one containing α′ and α subunits. These fractions were employed to immunize the mice, and after four immunizations two

sera: anti-αα′ and anti-β were obtained. With the aim of characterizing the specificity of these antibodies different soybean protein preparations (soybean protein isolate, crude 7S fraction, αα′ and β subunit purified fractions) were separated by SDS-PAGE and their reactivity was analysed by immunoblotting. Anti-αα′ and anti-β sera recognized mainly β-conglycinin subunits but not A and B polypeptides of glycinin (Figure 1 lane 1, part A vs. part B and C). Anti-αα′ serum reacted principally with α and α′ subunits: both subunits were similarly recognized (Figure 1, part B, lanes 1, 2 and 3 vs. Part A, same lanes). β′ Subunit was also recognized by anti-αα′ serum (Figure 1 lane 2, Part B vs. A). Anti-β serum mainly recognized β-subunit (Figure 1, part C vs. part A) and β′ subunit (Figure 1, part C lane 2) and presented a weak recognition of α and α′ subunits.

In order to confirm that the band identified as β′ subunit in Figure 1 was the same that has been previously described by Coates et al. (1985) and not the degradation product of α subunit described by Morita et al. (1996), we decided to analyse its chromatography behaviour. With this aim, a soybean protein isolate was separated using Q-Sepharose chromatography under dissociating conditions (Figure 2A). The chromatographic profile obtained had four peaks: peak 1 contained β′ subunit and other low molecular weight proteins (Figure 2B, lane 1); peak 2, β subunit (Figure 2B, lane 2); peak 3, α′ subunit (Figure 2B, lane 3) and peak 4, α and α′ subunits (Figure 2B, lane 4). Therefore, the protein labelled as β′ subunit interacts weakly with the anion exchange gel similar to the behaviour reported by Coates et al. (1985). α Subunit degradation product described by Morita et al. (1996) was not characterized by chromatography in denaturing conditions, but was observed only in some soybean varieties, that had a protease that is activated at pH 6.4 during the precipitation of crude 11S fraction previously to the precipitation of the 7S fraction at pH 4.8. To avoid this specific proteolytic activity at pH 6.4 instead of using a crude 7S fraction, we purified by chromatography a soybean protein isolate, obtained in the presence of a protease inhibitor cocktail. Based on the chromatographic behaviour shown above, we consider that the protein, labelled as β′ subunit in this work, is the

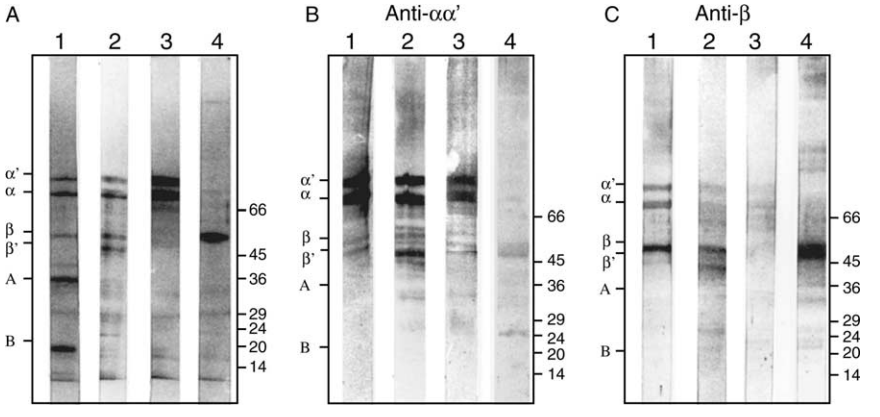

Figure 1. SDS-PAGE in a 5–15% polyacrylamide linear gradient (part A) and the corresponding blottings developed with anti-αα′ serum (part B) and with anti-β serum (part C). Lane 1, soybean isolate; lane 2, crude 7S fraction; lane 3, αα′ purified fraction; lane 4, β purified fraction. Proteins identified on the left: α′ α, β and β′ subunits of β-conglycinin; A and B polypeptides of glycinin. Molecular weights in kDa are indicated on the right.

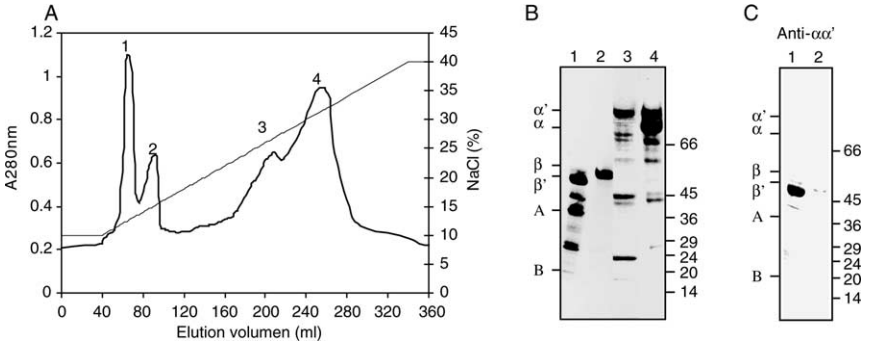

Figure 2. Analysis of soybean protein isolate by anion exchange chromatography under denaturing conditions. Part A shows the chromatographic profile obtained when elution is performed with the NaCl concentration indicated by the grey line. SDS-PAGE of the fractions indicated at the elution profile are shown in Part B; and the corresponding blottings developed with anti-αα′ serum in Part C. Peak 1 contains β′ subunit and other low molecular weight proteins; peak 2, β subunit; peak 3, α′ subunit; peak 4, α′ and α subunits. Proteins identified on the left: α′, α, β and β′ subunits of β-conglycinin; and A and B polypeptides of glycinin. Molecular weights in kDa are indicated on the right.

same that was described by Coates et al. (1985). To continue with the characterization, the first two peaks of this chromatography were analysed by immunoblotting. Anti-αα′ serum strongly recognized β′ subunit (Figure 2 lane 1, part B vs. part C), but did not recognize β subunit (Figure 2 lane 2, part B vs. part C). Therefore β′ subunit primary sequence is apparently more similar to α and α′ subunits than to β subunit.

To further characterize these sera, the presence of cross-reacting epitopes in these subunits was evaluated by competitive ELISA. Figure 3, part A shows the binding of anti-αα′ serum to αα′-subunit coated wells, when this serum was preincubated by either αα′ or β subunits. Binding of anti-αα′ serum, to the wells, was strongly inhibited when competition was performed with αα′ subunits. On the contrary, no competition was observed when β-subunit was used to inhibit antibody from binding to αα′

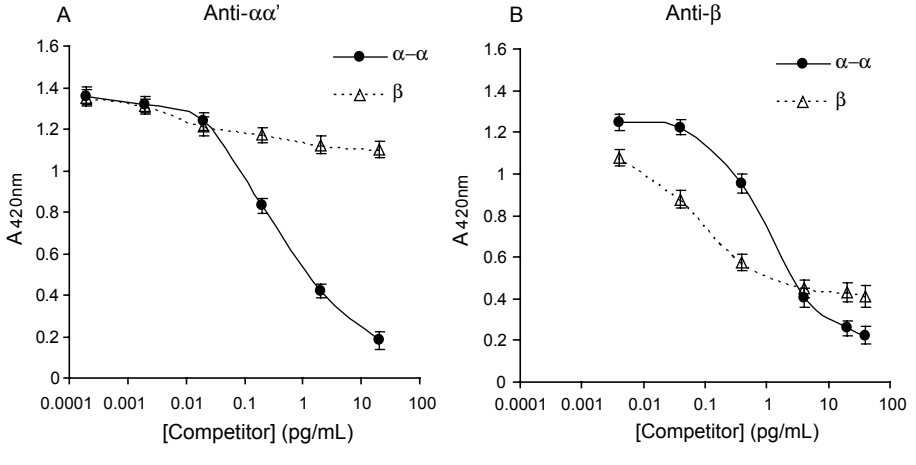

Figure 3. Analysis of the cross-reactivity between αα′ subunits and β-subunit. (A) Binding of anti-αα′ serum to αα′ subunits coated wells, when the serum is preincubated with αα′ subunits (solid circles) or β-subunit (empty triangles). (B) Binding of anti-β serum to β subunit coated wells, when the serum is preincubated with αα′ subunits (solid circles) or β-subunit (empty triangles). Points are means of three independent experiments and vertical bars represent the standard deviation.

subunits coated wells (Figure 3A). This result agrees with that observed by immunoblotting (Figure 1). In contrast, when anti-β serum reactivity was analysed, both αα′ and β subunits were able to compete and inhibit the binding of this serum to β subunit coated wells. Nevertheless, there is an indication of higher apparent affinity of anti-β for β subunit than for αα′ subunits, because the inhibition of binding occurs at a lower protein concentration. This difference in the apparent affinity of anti-β serum is also stated when competition index is calculated; the amount of αα′ subunits required to produce a 50% decrease in the maximum absorbance is ten-fold higher than that of the β subunit.

Reactivity of the sera with recombinant fractions

To further characterize the reactivity of the anti-β and anti-αα′ serum, the full-length cDNA encoding for alpha subunit and a deletion mutant named αCTD (C-terminal domain) were cloned and their sequences were confirmed. A schematic representation of α, α′, β and αCTD is shown in Figure 4. β-Conglycinin subunits have a core region that is formed by two domains (N and C terminal domains, shown in grey and dark-grey, respectively) with the same structure (β-barrel followed by α helix region, shown in black). In addition to these domains, α and α′ subunits have at the N terminus a

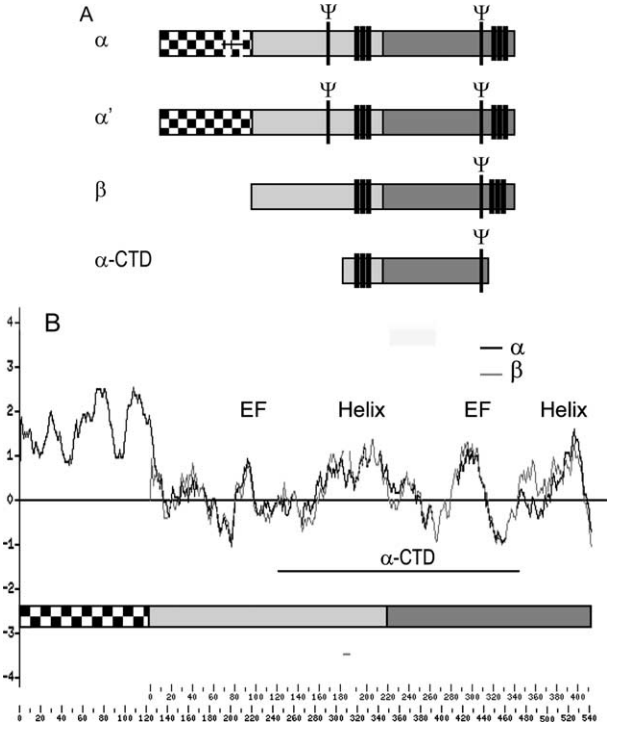

Figure 4. (A) Schematic diagram of mature beta-conglycinin subunits and α-C-terminal domain (α-CTD). Extension region, N and C terminal domains are presented in squared, grey and dark-grey boxes, respectively. Helix regions at the N and C terminal domains are shown in black. Ψ represents glycosylation sites. (B) Hydropathic profile of α and ß subunits using the Hopp-Woods method calculated by the Bioinformatics and Biological Computing Unit, Weizmann Institute of Science, Israel (http://bioinformatics.weizmann.ac.il). EF loop and Helix region of the N and C terminal domains are indicated.

region named extension region (shown as squared box). Hydropathic profile of α and β subunits using the Hopp-Woods method is shown in Figure 4B (Hopp & Woods 1981). This analysis reveals that the extension region has the highest hydrophilicity, and that both N and C terminal domains have two highly hydrophilic regions, which based on the β-conglycinin three dimensional structure, correspond to the loop between E-F β strands and α helix region (Maruyama et al. 2001). αCTD mutant has a deletion of the extension, of the N-terminal domain β-barrel and of the C-terminal domain helix regions (Figure 4A). The full-length mature α subunit and αCTD mutant were cloned fused to a His tag at the N terminal, and the obtained plasmids were introduced into *E. coli* BL21 for expression. Both mature α subunit and αCTD were produced in *E. coli* as inclusion bodies, which were nickel affinity purified to obtain α-CTD and α subunit purified fractions. Recombinant α subunit and α-CTD were analysed by immunoblotting with anti-αα′ and anti-β sera (Figure 5B and C, respectively). To compare visually the immunochemical reactivity among these subunits, the same amount of each protein was loaded into the gel electrophoresis (Figure 5A). Anti-αα′ serum strongly reacted with the full-length recombinant α subunit, but it had no reactivity against α-CTD (Figure 5B, lanes 1, 2, respectively). Anti-β serum presented good recognition of soybean β subunit and also recognized the full-length recombinant α subunit, but it did not recognize α-CTD (Figure 5C). The reactivity of both sera against recombinant fractions is different. For example, anti-αα′ serum strongly recognized both recombinant and soybean purified α subunit (Figure 5 lane 2 and 4, A vs. B); in contrast anti-β serum strongly recognized recombinant α subunit but has a weak reactivity against soybean purified α subunit (Figure 5 lane 2 and 4, A vs. C). Neither anti-αα′ serum nor anti-β serum recognized α-CTD.

Discussion

Soybean proteins are utilized in many food products; however, many people suffer from adverse reaction, such as allergy. For that reason the development of immunodetection methods against soybean proteins is an important issue. Knowledge about immunoreactivity of soybean proteins is scarce and such information is valuable to develop methods for quantitative determination of specific soybean proteins. In this work, sera against soybean purified αα′ subunits and β subunit were generated in

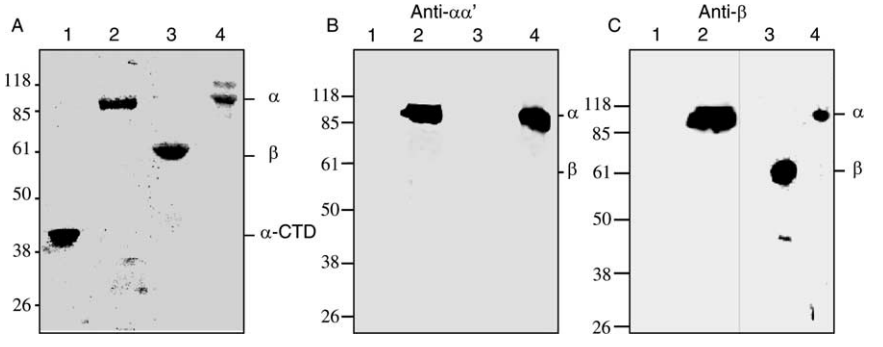

Figure 5. SDS-PAGE 10% polyacrylamide (part A) and the corresponding blottings developing with anti-αα′ serum (part B) and with anti-β serum (part C). Lane 1, recombinant α-CT domain; lane 2, recombinant α subunit; lanes 3 soybean purified β subunit; lane 4, soybean purified αα′ subunits. Proteins are identified on the right. Molecular weights in kDa are indicated on the left.

mice and their reactivity with soybean and recombinant proteins was analysed. Our results show that anti-αα′ serum mainly recognizes α and α′ subunits, but the β subunit is unable to compete with αα′ subunits, despite the high homology degree that exhibits the core regions among α, α′ and β subunits (Maruyama et al. 1998). The lack of cross reactivity of anti-αα′ serum with β subunit suggests that the main antigenic determinant of αα′ subunits is the extension region; and that the N and C terminal core domains are very weak immunogens, when the entire αα′ subunits are used as antigens. These results agree with to those published by Plumb et al. (1995), who produced monoclonal antibodies against the whole β-conglycinin and found that such monoclonal antibodies were all reactive against the extension region. On the contrary, when serum is raised against the core region using ß subunit as antigen, that exhibits 75.5% homology with α subunit, cross reactivity of anti-β serum with αα′ subunits is observed.

Although anti-αα′ serum was not reactive against β subunit, we found that this serum strongly recognized β′ subunit (Figure 2). β′-Subunit was first reported by Coates et al. (1985) who named this subunit, β′ based on its chromatographic behaviour and amino acid composition, which are similar to β subunit – although the methionine content of β′ subunit is higher than that of β subunit. The sulphur-rich amino acid content in β′ subunit makes it very interesting from a nutritional point of view. β′-subunit was further characterized by Morita et al. (1996) but its primary sequence remains unknown. Since in the present study β′ subunit is strongly recognized by anti-αα′ serum; while this serum showed no reactivity against β subunit, we speculate that β′ subunit primary structure should be more similar to α and α′ subunits than to β subunit. Based on that anti-αα′ serum mainly recognizes the extension region, a similar region should be present in β′ subunit.

As mentioned previously, the core region of β conglycinin subunits like other 7S storage globulins and 11S storage globulins, are composed of two similar domains (Ko et al. 1993; Lawrence et al. 1994; Adachi et al. 2001; Maruyama et al. 2001) that have evolved from a common single-domain ancestor (Shutov et al. 1996). This single domain is formed by a β-barrel with a 'swiss' or 'jelly' roll folding motif, followed by an α helix domain comprising three helices, two of which exhibit the helix-turn helix motif (Ko et al. 1993; Lawrence et al. 1994; Adachi et al. 2001; Maruyama et al. 2001). The β-barrel structure is termed 'cupin' (cupa is the Latin name for small β-barrel) because the loops connecting antiparallel β strands are very short consequently are not very exposed (Dunwell et al. 2000, 2001). Potential antigenic epitopes frequently are hydrophilic, surface-oriented and flexible (Van Regenmortel 1986), for that reason it is expected that within each domain, the helix region should be more immunogenic than the cupin β barrel. In addition, the immunoreactivity of the N and C terminal domains is also expected to be comparable because they have essentially the same structure. To analyse the hypothesis that the N and C terminal domains have similar immunoreactivities, we used anti-β serum that was generated against the core region and two recombinant proteins: the entire α subunit and deletion mutant (α-CTD). The latest has a deletion of the extension region, N terminal-β barrel and C terminal-helix region (Figure 4). The N terminal helix region was kept on this mutant because this region has been described as the main allergenic epitope of α subunit (Ogawa et al. 1995). From the results presented in this work, with the recombinant fractions, we can make the following interpretation. First, although β-conglycinin subunits are glycosylated, recombinant fractions were

recognized by both anti-αα′ and anti-β sera, therefore, glycosylation is apparently not necessary for recognition by these sera to occur. Second, the serum raised against the core region (anti-β serum) strongly recognizes β subunit and recombinant α subunit. Third, α-CTD protein has no reactivity with anti-αα′ serum and anti-β serum. The former result is expected since anti-αα′ serum recognizes the extension region that is not present in α-CTD protein. On contrary, if the N and C terminal domains present an equivalent immunoreactivity, α-CTD protein is expected to be recognized by anti-β serum. Considering that anti-β serum strongly recognizes recombinant α-subunit but has no reactivity against α-CTD recombinant protein, the region of α-subunit that is recognized by this serum should be localized at N terminal β barrel region (probably E-F loop) or C-terminal α-helix region (Figure 4). This result suggests that although both N and C terminal domains were present in β subunit used to immunize, they were not apparently equivalent as antigens. Remarkably, the N terminal helix region, which is the main allergenic epitope of α subunit and also G2 glycinin (Ogawa et al. 1995; Xiang et al. 2002), does not interact with anti-β serum. According to these findings there is no correlation between immunogenicity and allergenicity. Interesting, the differences in the immunochemical behaviour between the N and C terminal domain shown in this work, are not reflected when exposed regions of β conglycinin subunits are analysed by limited proteolysis (Shutov et al. 1996). It is also important to notice that recombinant α subunit is strongly recognized by anti-β serum, while recognition of soybean purified α subunit is weak. This result should take into account when recombinant proteins are used to standardize immunoassays to avoid natural variation produced when different soybean cultivars are used or when protein components or posttranslational processing are affected by changes in plant growing conditions.

In conclusion, our experimental data indicates that the main immunoreactive region of α and α′ subunits, and probably also β′ subunit is the extension region. If this region is present in the antigen, mainly all antibodies will recognize this region and not the core region. Within the core region, although the N and C terminal domain are structurally equivalent their immunoreactivity is different. Finally, the reactivity of recombinant fractions was found to be different from soybean purified fractions. Analysis of the reactivity of soybean purified β conglycinin subunits and αCTD protein with sera of soybean allergic patients is under progress.

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