



Carbohydrate moieties on the *in vitro* immunoreactivity of soy β -conglycinin

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ABSTRACT

β -Conglycinin is a functional glycoprotein and one of the most important soybean allergens. The aim of the present research was to investigate the role of the *N*-glycans moieties of β -conglycinin on its *in vitro* immunoreactivity. The soy allergen was obtained by isoelectric precipitation from commercial soy protein isolate and was enzymatically deglycosylated by PNGase F (Peptide *N*-Glycosidase F EC 3.5.1.52). In order to optimize deglycosylation conditions different reaction times and allergen concentrations were tested. The extent of deglycosylation was estimated by SDS–PAGE, CZE, RP–HPLC, and MALDI–TOF MS analyses, which provided information related to changes in protein structure. The antigenicity of both native β -conglycinin and its deglycosylated form was evaluated by western-blotting and indirect ELISA employing polyclonal rabbit *anti*-soybean sera and horseradish peroxidase-labeled goat *anti*-rabbit IgG while the *in vitro* allergenicity was assessed by means of indirect competitive inhibition ELISA employing human sera (IgE) of soy allergics. β -Conglycinin was effectively deglycosylated by PNGase F. Data on immunological tests suggested that glycosyl moieties forming this glycoprotein might be involved in its immunoreactivity.

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1. Introduction

Soy β -conglycinin represents about 85% of the 7S soy protein fraction (L'Hocine & Boye, 2007). The 7S fraction of soybean constitutes around 33% (w/w) of the total soy protein. β -Conglycinin has recently started to be commercialized as functional food ingredient (http://www.fujiol.co.jp/fujiol_e/product/soy_index6.html).

However, β -conglycinin has been also identified as one of the major soy allergens being recognized by about 25% of sera from soybean-sensitive patients (Ogawa et al., 1991).

β -Conglycinin is a trimeric glycoprotein with a molecular weight of 150–210 kDa. Its subunits, α (57–76 kDa), α' (57–83 kDa), and β (42–53 kDa) (García, Torre, Marina, & Laborda, 1997) can be combined forming homotrimers or heterotrimers ($\alpha\alpha\alpha$, $\alpha\alpha\alpha'$, $\alpha\alpha\beta$, $\alpha\beta\beta$, $\alpha\alpha'\beta$, $\alpha\beta\beta$, $\alpha'\beta\beta$) (L'Hocine & Boye, 2007). β -Conglycinin quaternary structure is assembled by hydrophobic forces and hydrogen bonds (Thanh & Shibasaki, 1977). Carbohydrate moieties constitute around 5% of the glycoprotein and are *N*-linked to the polypeptide chains (Yamauchi, Kawase, Kanbe, & Shibasaki, 1975). The carbohydrate contains an *N*-acetylglucosamine residue at its reducing terminal which is linked to the amide group of an asparagine residue of the polypeptide (Kobata, 1992). The subunits called α and α' contain two consensus sequences of

glycosylation while the β subunit has only one (Thanh & Shibasaki, 1977). Little information related to the exact structure of the glycans attached to β -conglycinin has been published (Kimura, Ohno, & Takagi, 1997; Yamauchi et al., 1975).

Several *N*-linked glycan moieties have been described as epitopes of glycoprotein allergens (Fötisch & Vieths, 2001; van Ree, 2002) or as responsible for cross reactivity with other allergens (Rodríguez & Villalba, 1997). Soy glycoproteins have not been completely characterized regarding to their immunological properties and the allergenic role of their glycan moieties is practically unknown. Up to date, only three papers dealing with this issue have been published (Babiker, Azakami, Ogawa, & Kato, 2000; Fu, Jez, Kerley, Allee, & Krishnan, 2007; Hiemori et al., 2000). The present research aimed to find out an effective method for removing all intact *N*-linked oligosaccharides from the trimeric glycoprotein and to evaluate the impact of the deglycosylation on *in vitro* immunoreactivity of β -conglycinin.

2. Materials and methods

2.1. Purification and deglycosylation of β -conglycinin

β -Conglycinin was purified from soy protein isolate (Manuel Riesgo, S. A., Madrid, Spain) following the method of Nagano, Hirot-suka, Mori, Kohyama, and Nishinari (1992). The obtained protein fraction was washed twice with distilled water, freeze-dried and

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stored at -20°C until analysis. The protein content was measured by Biuret method (Boyer, 2000). The purity grade of β -conglycinin was estimated by SDS–PAGE.

Pure β -conglycinin was deglycosylated employing proteomic grade PNGase F (Peptide N-Glycosidase F EC 3.5.1.52, P7367, Sigma, Saint Louis, MO, USA). In general, deglycosylation was carried out following the procedure of the manufacturer with small modifications. Briefly, 90 μL of β -conglycinin were denatured prior to deglycosylation by addition of 5 μL 2% octyl- β -D-glucopyranoside (Sigma, Saint Louis, MO, USA) and 0.1 M β -mercaptoethanol (Merck, Hohenbrunn, Germany) followed by heating at 100°C for 10 min. Five microlitres of 20 mM ammonium carbonate buffer, pH 7.8 and 4 μL of PNGase F equivalent to 2U of enzyme (test samples) or distilled water (negative control) were added to denatured glycoprotein samples. Samples were incubated in a Thermomixer (Eppendorf, Hamburg, Germany) at 37°C for 24 h with constant stirring at 300 rpm. After deglycosylation, the enzyme was inactivated by heating at 100°C for 10 min. In order to obtain optimal deglycosylation conditions the variables reaction time and glycoprotein concentration were studied. Reaction times from 1 to 24 h, and concentrations of β -conglycinin from 1 to 6 mg/mL were assessed. β -Conglycinin deglycosylation was followed by SDS–PAGE, CZE, RP–HPLC and MALDI–TOF MS analyses. All the analyses were performed at least in duplicate.

2.2. Structural characterization of deglycosylated β -conglycinin

2.2.1. SDS–PAGE

Samples containing 1 mg protein/mL (32.5 μL) were mixed with 5 μL of DTT (0.5 M) and 12.5 μL of NuPAGE[®] LDS sample buffer (4X) (Invitrogen, Barcelona, Spain). Thirteen microgram of denatured protein were loaded onto the gel. Separation was performed on a 12% polyacrylamide NuPAGE[®] Novex Bis–Tris pre-cast gels employing a continuous buffer system (NuPAGE[®] MES SDS running buffer, Invitrogen) for 50 min, initial current of 120 mA/gel and constant voltage of 200 V. A molecular weight marker with proteins ranging from 2.5 to 97 kDa was applied to the gel. Protein subunits were detected by Coomassie blue (Boyer, 2000) and periodic acid Schiff (PAS) (Zacharius, Zell, Morrison, & Woodlock, 1969) staining methods. Coomassie blue stained polypeptide chains while PAS stained carbohydrate moieties attached to β -conglycinin.

2.2.2. CZE analysis

The CZE method was based on that of Wong, Carey, and Lin (1994). A G1600A (Agilent, Madrid, Spain) capillary electrophoresis instrument equipped with a ChemStation software was used. Capillary zone electrophoresis was performed on uncoated fused silica capillaries 64.5 cm long (56 cm to the detector, respectively) with an internal diameter of 50 μm and a $\times 3$ bubble cell. Other conditions of analyses were as follows: buffer, 20 mM sodium carbonate at pH 8.5; voltage, 20 kV; temperature of analysis, 30°C ; injection for 5 s at 5 bar; electroosmotic flow (EOF) marker, dimethylsulfoxide. Electropherograms were monitored at 220 nm. The capillary was conditioned after each sample run by flushing water for 1 min, 0.1 M NaOH for 1 min, water for 1 min and 20 mM sodium carbonate pH 8.5 for 2 min at 5 bar.

2.2.3. RP–HPLC analysis

The RP–HPLC system consisted of a Beckman binary gradient 125 pump HPLC (Beckman, Fullerton, CA, USA) equipped with a Metrohm–Spark Triathlon autosampler (Herisau, Switzerland) and a Beckman 166–UV detector. A System Gold 711 version software was used for data acquisition. Chromatographic conditions were as previously described by Amigo-Benavent, Silván, Moreno, Villamiel, and del Castillo (2008). Separations were performed on a Phe-

nomenex Jupiter 300 Å (C_4 , 5 μm particle size, 250×4.60 mm i.d.) employing a linear binary gradient from 35% to 70% mobile phase B in 45 min at room temperature. Mobile phases were: phase A, 0.025% (w/v) SDS and 0.1% (v/v) TFA in Milli-Q water; phase B, 0.025% (w/v) SDS and 0.085% (v/v) TFA in Milli-Q water: acetonitrile (10:90, v/v) (Lab-Scan Analytical Sciences, Dublin, Ireland). The flow rate was 1 mL/min, 100 μL was injected and UV detection was performed at 214 nm.

2.2.4. Matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI–TOF MS) analysis

MALDI–TOF MS experiments were carried out on a PerSeptive Biosystems (Framingham, MA) Voyager DE-PRO instrument equipped with a N_2 laser (337 nm, 3 ns pulse width). Each spectrum was taken with the following procedure: 1 μL -aliquot sample solution was loaded on a stainless steel plate together with 1 μL of sinapinic acid (10 mg in 1 mL of water) matrix. Mass spectrum acquisition was performed in the positive linear mode by accumulating 200 laser pulses. The accelerating voltage was 20 kV. External mass calibration was performed with high-mass protein standards (PerSeptive Biosystems, Framingham, MA).

2.3. Immunoreactivity of β -conglycinin

2.3.1. Western-blotting for in vitro antigenicity evaluation

After electrophoresis (see Section 2.2.1) proteins were electroblotted onto a 0.45 μm Invitrolon[™] PVDF membrane using the XCell II Blot module (Invitrogen), at 30 V for 2 h, following the manufacturer's instructions. Blots were blocked with TBST [0.05% (v/v) Tween-20 in Tris-buffered saline (TBS)] containing 10% (w/v) milk powder for 1 h at room temperature. After washing with TBST, blots were incubated at 4°C overnight with a polyclonal rabbit anti-soy sera diluted 1:3000 (v/v) in TBST, washed and incubated with a horseradish peroxidase-labeled goat anti-rabbit IgG (Sigma, Steinheim, Germany) diluted 1:1000 (v/v) in TBST containing 10% (w/v) milk powder for 1 h at room temperature. Blots were again washed five times with TBST and detected with 3,3',5,5'-Tetramethylbenzidine (TMB, Sigma).

2.3.2. ELISA

Prior to analysis carbohydrate moieties cleaved by PNGase F were removed from the deglycosylation reaction mixture by ultrafiltration using Centricon YM-10 centrifugal filter devices (Millipore, Bedford, MA) at 5000g for 30 min. The retentate was washed twice with distilled water and the final protein concentration determined by Biuret method (Boyer, 2000).

2.3.2.1. Indirect ELISA for studying in vitro antigenicity. The wells of a 96-well ELISA Polystyrene High Bind microplate (Costar 3590, New York, USA) were coated with antigens (native and deglycosylated β -conglycinin) and the plate was incubated at 4°C overnight. The remaining protein-binding sites in the coated well were blocked by adding 300 μL of 1% (w/v) BSA in 50 mM sodium carbonate buffer, pH 9.5 at 37°C for 2 h. The plate was washed three times with PBS–Tween (PBST) buffer (phosphate saline buffer [50 mM NaCl, 0.15 mM H_2KPO_4 , 0.2 mM HNa_2PO_4 and 0.3 mM KCl] pH 7.2, and 0.05% (v/v) Tween-20) to remove the excess of BSA. Diluted polyclonal rabbit anti-soy IgG serum (1000-fold in PBS) was added to each well and the plate was incubated at 37°C for 2 h. Subsequently, the plate was washed three times with PBST to remove the excess of primary antibody. One hundred microlitres of horseradish peroxidase-labeled goat anti-rabbit IgG (Sigma) diluted 4000-fold in PBS were added (secondary antibody) and incubated at 37°C for 1 h. The plate was washed three times with PBST to remove the excess of secondary antibody. Detection of the antigen was performed by adding 100 μL of chromogen solution TMB to

each well and incubating in darkness at room temperature for 10 min. The colorimetric reaction was stopped by adding 100 μ L 2 M HCl and the absorbance values were measured at 450 nm with a plate reader (Biotek Power Wave XS, Izasa, Madrid, Spain). Since a linear relationship between concentration of antigen and absorbance at 450 nm was found by analysis of pure β -conglycinin ranging from 10 to 80 ng/well a concentration of 40 ng/well of sample in any case was chosen for analysis. The percentage of antigenicity of the samples was calculated by using this formula: $(\text{Abs}_{\text{sample}}/\text{Abs}_{\text{control}}) \times 100$. $\text{Abs}_{\text{sample}}$, is the absorbance value of any tested sample and $\text{Abs}_{\text{control}}$ the absorbance value obtained by analysis of pure β -conglycinin. All measurements were carried out in triplicate.

2.3.2.2. Competitive inhibition ELISA (ciELISA) for assessing in vitro allergenicity. The characteristics of the human sera used in the immunological experiments, purchased from PlasmaLab International (Everett, WA), are presented in Table 1. A pooled plasma sample of equal volumes of five sera of soy allergics was used. A pool of five sera from nonatopic individuals was used as blanks. The assay was carried out using similar protocol with that of indirect ELISA with the following modifications. The primary antibody was a pool of human sera diluted (50-fold in PBS); the secondary antibody was goat-antihuman IgE peroxidase diluted 1000-fold in PBST. The plate was coated with native β -conglycinin (150 ng/well) and blocked as described above. Inhibitor (β -conglycinin or its deglycosylated form) was mixed with primary antibody to final concentrations ranging from 10 to 50 ng/mL of inhibitor and 1:50 primary antibody. The mixture was immediately added to the plate (100 μ L/well) and incubated for 2 h. The plate was washed followed by detection with goat-antihuman IgE peroxidase and TMB. The % inhibition was calculated by using the formula $100 - 100(A_2 - A_1)/(A_0 - A_1)$. A_0 is the absorbance in the absence of inhibitor (maximum absorbance); A_2 is the absorbance at any given concentration; A_1 is the average absorbance of the control wells that do not contain any primary antibody (minimal signal). All measurements were carried out in triplicate.

2.4. Statistical analysis

Microsoft Excel 2000 Program was employed for statistical analysis of the data with the level of significance set at 95%. Student's *t*-test (means comparison) was used to look for differences between two groups of means.

3. Results

3.1. Optimal conditions for effective deglycosylation of β -conglycinin

Fig. 1 shows SDS–PAGE patterns of β -conglycinin and its deglycosylated forms obtained by treatment at different deglycosylation times (A and C) and glycoprotein concentrations (B and D). In Fig. 1A and B can be clearly observed the three typical electrophoretic bands with molecular weights of about 68,000, 64,000 and 49,000 Da of β -conglycinin. Moreover, a protein band with a molecular weight of $\sim$ 34,000 Da was detected.

The electrophoretic pattern of β -conglycinin was modified after treatment with PNGase F. The polypeptides bands found in deglycosylation reaction mixtures showed slightly lower molecular weight and consequently higher electrophoretic mobility than the corresponding control samples (Fig. 1A and B). Bands of glycoproteins were not detected in the deglycosylation mixtures by SDS–PAGE stained with PAS (Fig. 1C and D) suggesting that all the oligosaccharides linked to the polypeptide chain of β -conglycinin in concentrations from 1 to 6 mg/mL were cleaved by activity of 2U of PNGase F for 24 h. Partial deglycosylation of β -conglycinin subunits (1 mg/mL) was achieved by enzymatic activity for 2 h (data not shown); however, 3 h of treatment resulted effective to achieve this goal.

In order to add further support, CZE, RP–HPLC, and MALDI–TOF MS analyses of the deglycosylation test mixtures obtained after 24 h of enzymatic activity and the corresponding control were performed. Fig. 2 illustrates the variation of CZE mobility of β -conglycinin due to its enzymatic deglycosylation. Protein peaks corresponding to native β -conglycinin (8.50 min) and its deglycosylated form (9.25 min), migrated after a neutral electroosmotic flow marker (5.5 min) suggesting that they were negatively charged migrating toward the anode and showed similar shape.

RP–HPLC profiles of β -conglycinin and its deglycosylated form are shown in Fig. 3. Relatively sharp and symmetric protein peaks were obtained by application of this analytical approach. Retention times of 32.0 and 33.1 min were found for the native β -conglycinin and the corresponding deglycosylated form, respectively. In RP–HPLC, compounds are separated based on their hydrophobic character. Therefore, data suggested that the hydrophobic character of the soy glycoprotein was increased by PNGase F action. Data on RP–HPLC agreed with those on CZE and SDS–PAGE indicating a modification of the protein structure due to enzymatic activity.

MALDI–TOF MS spectra relative to the analysis of β -conglycinin prior (A) and after (B) enzymatic deglycosylation are shown in Fig. 4. In the native sample, three main components can be observed in the high-mass region of the spectrum at 67,209, 65,289 and 48,785 Da, which were assigned to the α' , α and β subunit, respectively. As can be seen from Fig. 4B, the molecular mass of the subunits was shifted to lower mass values as a consequence of PNGase F deglycosylation. In particular, the protein mass of α' subunit changed from 67,209 to 63,859 Da that of α from 65,289 to 61,912 Da and β from 48,785 to 47,194 Da, indicating the presence of larger carbohydrate moieties in α' (3350 Da) and α (3377 Da) subunit compared to the β subunit (1561 Da).

3.2. In vitro immunoreactivity

Fig. 5 shows the IgG immunoblotting of β -conglycinin and its deglycosylated form obtained by PNGase F activity. In agreement with the SDS–PAGE data above presented, different migration behavior was observed for the native (lane 1) and deglycosylated glycoprotein (lane 2). No visual differences in intensity of the protein bands were observed. Therefore, results suggested a similar antigenicity for the two samples, incubated without (lane 1) and with enzyme (lane 2), after reaction with polyclonal antibody (IgG) from rabbit immunized with total soy proteins.

Table 1
Characteristics of human plasma used in immunological experiments.

Plasma code	Gender	Age (years)	Soybean-specific IgE by immunoCAP analysis (kUA/L)
A	M	38	95.7
B	F	44	44.9
C	M	24	35
D	M	40	30
E	F	50	10

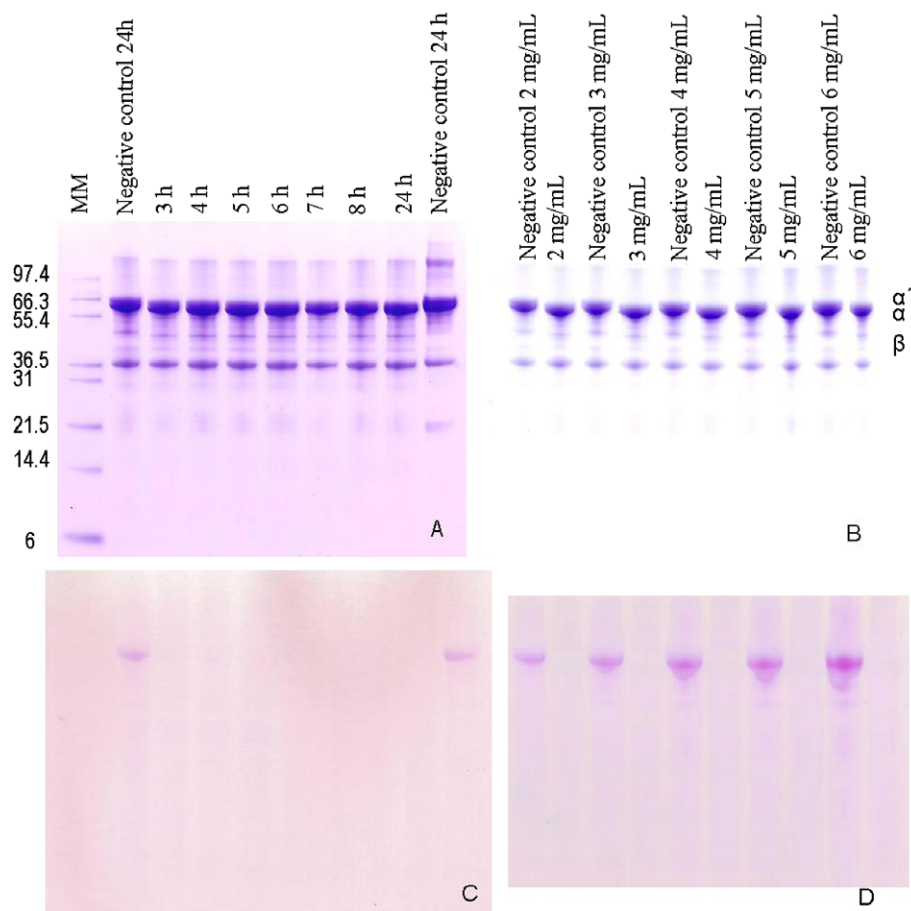


Fig. 1. SDS-PAGE patterns of pure soy β -conglycinin and its deglycosylated form obtained by activity of 2U of PNGase F employing different time of deglycosylation (A and C) and glycoprotein concentrations (B and D). Gels were stained employing either Coomassie blue (A and B) or PAS (C and D) staining methods. Mixtures no containing enzyme were considered as control. MM means molecular weight makers. The molecular weights of the proteins are given in kDa.

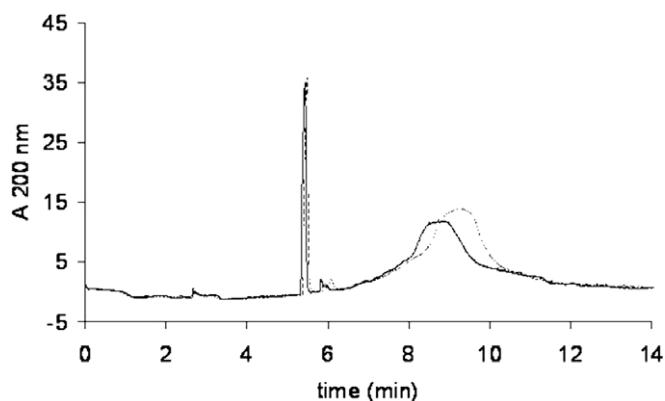


Fig. 2. CZE electropherograms of β -conglycinin (solid line) and its deglycosylated form (dash line).

In order to provide quantitative results on the role of glycosyl moieties of β -conglycinin on its antigenicity, the samples were analyzed by indirect ELISA. Data suggested that the antigenicity of deglycosylated β -conglycinin was 99% of that found for the native β -conglycinin. Solely a slight and statistically insignificant decrease in reactivity was detected.

In vitro allergenicity results obtained by competitive inhibition ELISA employing a pooled plasma sample of equal volumes of all five samples (primary antibody) and goat-antihuman IgE peroxi-

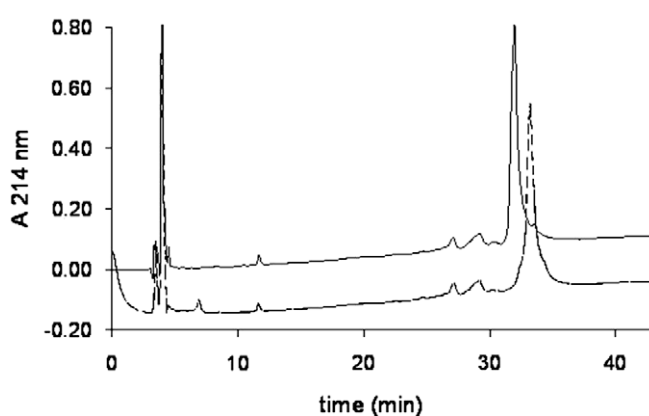


Fig. 3. RP-HPLC patterns of β -conglycinin (solid line) and its deglycosylated form (dash line).

dase (secondary antibody) are presented in Fig. 6. Results showed that the deglycosylated β -conglycinin exhibited a significant, lower inhibitory effect on IgE with IC_{50} value of approximately 10 μ g/mL, than the native β -conglycinin, which had an IC_{50} of less than 1 μ g/mL. The IC_{50} is defined as the concentration of proteins (inhibitors) required to inhibit IgE binding by 50%. The significant difference in IC_{50} indicated a 10-fold reduction in the *in vitro* allergenic response of the deglycosylated β -conglycinin.

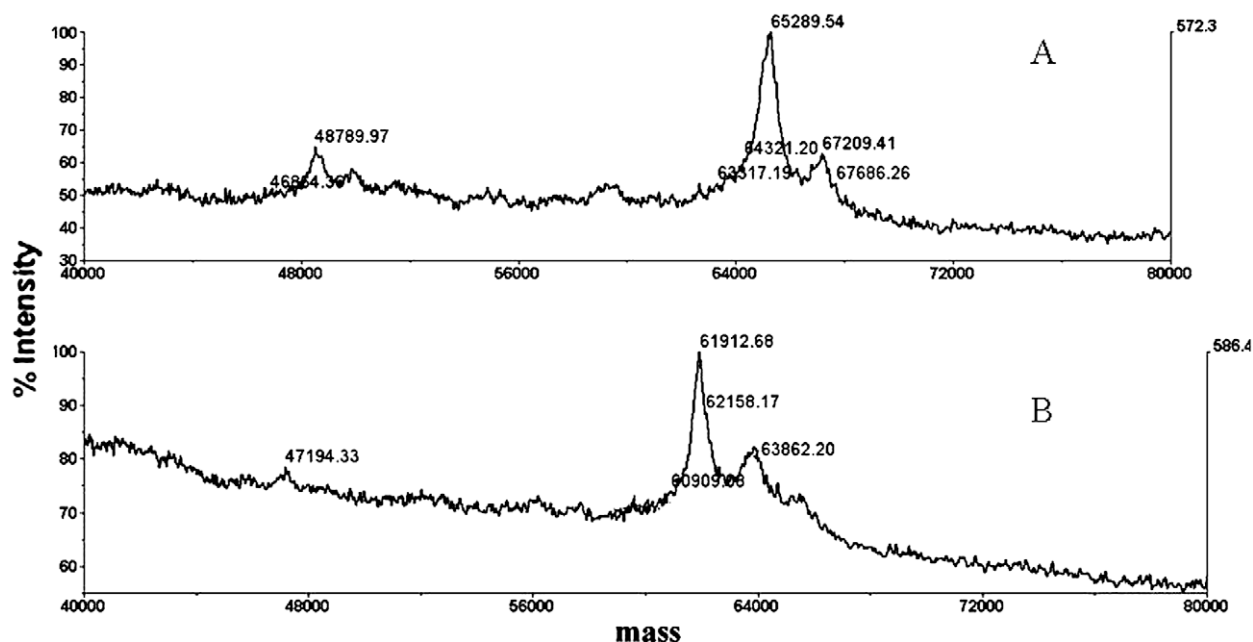


Fig. 4. MALDI-TOF mass spectra of β -conglycinin (A) and its deglycosylated form (B).

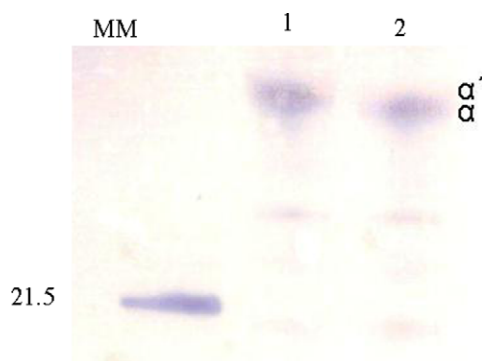


Fig. 5. IgG immunoblotting of β -conglycinin (lane 1) and its deglycosylated form (lane 2) obtained by incubation with commercial preparation of rabbit IgG developed against total soy proteins (primary antibody), horseradish peroxidase-labeled goat *anti*-rabbit IgG (secondary antibody) and staining with 3,3',5,5'-tetramethylbenzidine. Lane designed as MM shows the band corresponding to soybean trypsin inhibitor possessing molecular weight 21.5 kDa.

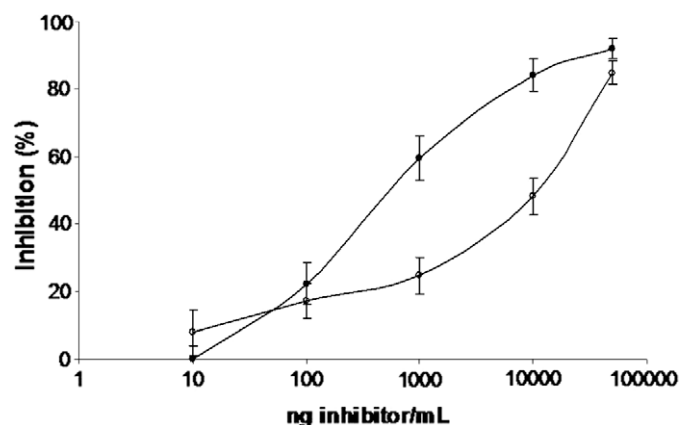


Fig. 6. Indirect inhibition IgE ELISA employing pooled plasma of five people allergic to soy. Data are expressed as mean values ($n=3$) and bars represent standard deviation. X-axis is logarithmic. Closed circle: native β -conglycinin, opened circle: deglycosylated β -conglycinin.

4. Discussion

4.1. Effective deglycosylation of β -conglycinin soy allergen

A highly pure fraction of soy β -conglycinin was obtained (Fig. 1). The bands detected corresponded well to those previously described as α , α' and β subunits of β -conglycinin (Garcia et al., 1997; Nagano et al., 1992). The observed band possessing lower molecular weight (~ 34 kDa) can be ascribed as P34. This protein may be attached to β -conglycinin (Garcia et al., 1997).

Electrophoretic, chromatographic and MS data confirmed the occurrence of structural changes of soy β -conglycinin by deglycosylation under the conditions here described. Since all *N*-linked oligosaccharides were cleaved by enzymatic activity of PNGase F (see Figs. 1–4) results seem to indicate that β -conglycinin does not contain *N*-linked glycan moiety with $\alpha(1-3)$ fucose structure (Tretter, Altmann, & Marz, 1991). PNGase F cleaves all asparagine-linked complex, hybrid, or high mannose oligosaccharides unless they were $\alpha(1-3)$ core fucosylated.

Shape of β -conglycinin CZE peak was similar to that obtained by Wong et al. (1994). The fact of the belt shaped peaks is probably due to protein adsorption to the fused silica capillary (Fig. 2). The protein can bind via electrostatic interactions with the ionized silanol groups on the capillary wall. Due to the high purity of the sample under analysis, differences in migration behavior ascribed to deglycosylation were observed. CZE separation was performed according to their number of glycosyl moieties linked to the polypeptide chain of β -conglycinin. CZE has been previously employed as a successful technique for studying intact glycoproteins and glycopeptides (Balaguer et al., 2006; Berkowitz et al., 2005; Weber, Kornfelt, Klausen, & Lunte, 1995; Yim, Abrams, & Hsu, 1995).

Data on RP-HPLC clearly supported the occurrence of an effective deglycosylation of β -conglycinin under the conditions proposed in the present paper since changes in its hydrophobic character have been observed after its enzymatic deglycosylation. RP-HPLC has been proved as feasible approach for characterization of glycopeptides from rFVIIa possessing the same *N*-glycosylation sites (Weber et al., 1995).

MALDI-TOF MS data agreed with those very recently reported by Horneffer, Foster, and Velikov (2007) (about 67,000, 64,000

and 47,000 Da for the α' , α and β subunit, respectively) employing this approach, and slightly differed with those reported in Swiss-Prot database which correspond to 67,240, 63,151 and 47,776 Da for α' , α and β , respectively (<http://www.expasy.org/cgi-bin/sprot-search-de?conglycinin>). This was not surprising considering that: (i) variations could be due to different cultivars employed that can generate diverse isoforms of the protein (Gianazza, Eberini, & Arnoldi, 2003); (ii) only DNA-deduced sequence are available in the database, which do not take into account the possible occurrence of transcriptional or post-translational processing events, including proteolysis or sequence deletions. These differences were not further investigated being beyond the aim of this study. The observed mass shift, corresponding to the molecular weight of the oligosaccharides previously linked to each protein subunit, were in full agreement with the few structures available in the literature (Kimura et al., 1997), although the detailed structure of the carbohydrate moieties is still largely unknown. MALDI-TOF MS data confirmed SDS-PAGE results previously obtained, unambiguously establishing quantitative deglycosylation of β -conglycinin isolate by PNGase F.

4.2. Role of glycosyl moieties on the in vitro antigenicity of β -conglycinin soy allergen

Our data on *in vitro* immunoreactivity suggested that the glycosyl moieties linked to β -conglycinin might contribute to the allergenicity of this glycoprotein. Differences in immunological reactivity against IgE due to deglycosylation were detected by competitive inhibition ELISA while evidences of changes in antigenicity (IgG response) were not found by employing neither western-blotting nor indirect ELISA.

The clinical relevance of carbohydrate specific IgE antibodies is still matter of controversial discussions. Several research groups have reported the existence of IgE antibodies against N-glycans on glycoproteins from plants and invertebrate animals (van Ree et al., 2000). Allergenicity of plant N-glycans has been shown to be caused by the presence of two typical nonmammalian substitutions which are $\alpha(1,3)$ -fucose linked to the proximal N-acetylglucosamine and $\beta(1,2)$ -xylose linked to the core mannose (Fötisch & Vieths, 2001; van Ree et al., 2000). According to our data on deglycosylation of β -conglycinin by PNGase F this glycoprotein does not contain $\alpha(1,3)$ -fucose structure.

Concerning N-glycans of soybean glycoproteins only several high mannose-type structures linked to soybean agglutinin (Dorland, Vanhalbeek, Vliegthart, Lis, & Sharon, 1981) and 7S globulin have been identified (Neeser, 1985). However, Kimura et al. (1997) found N-glycans structures having $\beta(1,2)$ -xylose residue in the soy glycoprotein that would form an allergenic epitope. Tsuji et al. (1997) also supported the presence of xylosyl and fucosyl residues in soybean allergens able to bind IgE from soybean-sensitive patients. IgE reactive N-glycosidic epitope is not a single uniform entity. Some glycoproteins contain both substitutions ($\alpha(1,3)$ -fucose and $\beta(1,2)$ -xylose), others just either one of them. Further studies should be conducted in order to confirm xylose presence in our isolated β -conglycinin. PNGase F may release high mannose-type and xylose-containing sugar chain (Tarentino & Plummer, 1994).

5. Conclusions

Our results suggested that the N-glycan structures of β -conglycinin might be involved in its immunoreactivity. Further researcher should be carried out to elucidate the specific role of these structures on the development of this undesirable biological response.

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