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Soybean basic 7 S globulin represents a protein widely distributed in legume species

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The LII subunit of the soybean (*Glycine max* (L.) Merrill) basic 7 S globulin seed storage protein was purified, and its amino acid sequence was determined. This subunit contains a relatively high proportion of sulphur-containing amino acids and an extremely low content of glutamic acid. The amino acid sequence was found to be homologous to that of conglutin γ from lupin seeds. Other legume species examined have a protein which cross-reacts with antibody raised against the LII subunit. This suggests that the basic 7 S globulin-like protein is widely distributed in the seeds of legume species.

Soybean; Seed storage protein; Basic 7 S globulin; Amino acid sequence; Immuno-blotting

1. INTRODUCTION

In the 7 S globulin fraction of the soybean seed storage protein, a novel basic protein, which has a higher isoelectric point (pH 9.05–9.26) than the other globulin species, was recently found and designated as basic 7 S globulin [1]. The basic 7 S globulin accounts for about 3% of the total seed protein [2]. This protein has a molecular mass of ~168 kDa and is composed of four pairs of high- and low-kDa subunits linked together via disulphide bond(s). The high- and low-kDa subunits each have two iso-forms, designated as HI and HII (~26 kDa) and LI and LII (~16 kDa) [3]. The basic 7 S globulin did not show immunological cross-reaction with the other globulin species of soybean [3].

In a previous paper [4], the N-terminal amino acid sequences of the HI/HII subunit (29 residues) and the LII subunit (15 residues) were reported. These sequences showed no homology with those of the other globulin species in soybean.

In the present study, we determined the amino acid sequence of the internal region of the basic 7 S globulin LII subunit and found that this sequence was homologous to that of the conglutin γ, one of the lupin seed storage proteins [5]. The cross-reaction of the seed proteins from 6 legume species with antiserum prepared against soybean basic 7 S globulin LII subunit suggested that the basic 7 S globulin-like seed storage protein is widely distributed in legume species.

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Abbreviations: HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid

2. MATERIALS AND METHODS

2.1. *Purification of the basic 7 S globulin*

The basic 7 S globulin LII subunit was partially purified from soybean (cv. Miyagishirome) seeds as described in [3]. The partially purified subunit (0.5 mg) was *S*-carboxymethylated [6] and suspended in 100 μl of 0.0625 M Tris-HCl buffer (pH 6.8) containing 10% glycerol, 5%

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2-mercaptoethanol and 2% SDS. The protein solution was heated at 95°C for 5–10 min to dissolve the protein and applied to a reverse-phase column (Nucleosil C8, 5 μm, 100 × 4.6 mm) for HPLC. The column was run at a flow rate of 1.5 ml/min and the subunit was eluted with a 20–100% acetonitrile gradient in 0.1% aqueous TFA for 50 min at a column temperature of 30°C. The effluent was monitored by absorbance at 216 nm.

2.2. *Amino acid sequence analysis*

The N-terminal amino acid sequence of the LII subunit (0.5 nmol) was analysed by a gas-phase protein sequencer (Applied Biosystems 470A) [7].

Portions (10 nmol) of the purified subunit were digested with trypsin, α-chymotrypsin, *Staphylococcus aureus* V8 protease, metalloendopeptidase from *Grifola frondosa* and cyanogen bromide as described in [8,9]. The digests were dissolved in 100 μl of 0.1% aqueous TFA, applied to a reverse-phase column (Nucleosil ODS, 5 μm, 250 × 4.6 mm) for HPLC and eluted at a flow rate of 1.0 ml/min with a 0–50% acetonitrile gradient in 0.1% TFA for 130 min at a column temperature of 30°C. The effluent was monitored by absorbance at 216 nm. The eluted peptides were collected, lyophilised and subjected to protein sequencing by the 470A sequencer.

2.3. *Preparation of antibody against the LII subunit and protein-blotting experiment*

Antibody against the LII subunit was raised in adult rabbits by the method described in [10]. The purified LII subunit solution containing Tris-HCl buffer as described above, and an equal volume of a complete adjuvant was injected into the rabbits three times at one month intervals. The antiserum obtained was used directly in the protein-blotting experiment.

The proteins extracted from the seeds of azuki bean, lupin, mung bean, pea and winged bean were separated by SDS-polyacrylamide gel electrophoresis [11] and were transferred and bound to a polyvinylidene difluoride membrane (Immobilon; Millipore) electrophoretically using a semi-dry blotting apparatus (Sartorius) [12]. The bands that cross-reacted with the antibody against the LII subunit were detected by peroxidase enzyme immunoassay (Bio-Rad immunoblot assay kit).

3. RESULTS AND DISCUSSION

The determined amino acid sequences of the peptide fragments of the LII subunit are shown in table 1. It should be noted that this subunit was insoluble in the buffer solutions normally used in protein digestion with cyanogen bromide and proteases. The cyanogen bromide and proteases except trypsin and α-chymotrypsin did not cleave the insoluble LII subunit. This makes the complete amino acid sequence determination extremely difficult. Although we could digest almost completely with trypsin and partially with α-chymotrypsin, we could not get overlapping peptides in a few regions.

In the sequence determined here, there are 8 methionines and 1 cysteine. This is in reasonable agreement with the results of amino acid analysis of the LII subunit [3]. This protein is particularly different from the other legume seed storage proteins like legumin and vicilin, which contain only low amounts of sulphur-containing amino acids [13].

According to the amino acid analysis [3], the LII subunit contains a high content of glutamic acid/glutamine. The acid and amide were not determined separately due to the destruction of amides after acid hydrolysis. The sequence obtained suggested that this subunit has an extremely low content of glutamic acid. This is considered to be one of the reasons why the basic 7 S globulin has a high isoelectric point, although the content of basic amino acids was low in the LII subunit.

The sequence of the LII subunit was compared with those of the other seed storage proteins of soybean such as glycinin [8,9,15], β-conglycinin [4,16], lectin [17] and trypsin inhibitors [18,20]. None of these proteins were structurally homologous to the LII subunit. Further comparison of the sequence of the LII subunit with sequences of the about 10020 proteins compiled in the amino acid sequence data base SEQDB (Protein Research Foundation, Osaka) showed that the conglutin γ, one of the lupin (*Lupinus angustifolius* L.) seed storage proteins, has a homologous sequence to the LII subunit. Fig.1 shows the comparison of the sequences of the LII subunit with that of the conglutin γ [5].

We also compared the physicochemical properties of the basic 7 S globulin and the conglutin γ

Table 1

The amino acid sequences of the tryptic (T1–T7) and chymotryptic (C1–C3) peptides from the basic 7 S globulin LII subunit

T1	S T I V G S T S G G T M I S T S T P X
T2	M V L Q Q S V Y Q A F X
T3	I V G P F G L C P N Q N G V T S L G P M X X M Q P A R
T4	Q L G L N L M V Q A Q P G V T X L G V M N G G M Q P R
T5	A E I T L G A R
T6	I N A P V (S) V D L E M D L P N G X
T7	G L S F N S N K I N A Y P S F D L A X
C1	N F A N A X
C2	N G G M Q P R A E I T L
C3	N S N K I N A Y

[21]. Both of the proteins have similar properties in solubility, molecular mass of subunits and subunit constitution.

Seed extracts of the following legumes were analysed for polypeptides similar to the soybean LII subunit: azuki bean (*Vigna angularis* L.), lupin (*L. albus* L. and *L. mutabilis* L.), mung bean (*Vigna radiata* L.), pea (*Pisum sativum* L.), and winged bean (*Psophocarpus tetragonolobus* (L.) DC), by a protein blotting experiment using antiserum obtained against the LII subunit. Although the antibody raised against the LII subunit did not cross-react with the protein extracted from pea seeds, a strong reaction was observed with the extracts from azuki bean and mung bean seeds, and a weak reaction with that of winged bean seeds (fig.2). According to the high degree of sequence homology between the LII subunit and the conglutin γ, cross-reaction between the conglutin γ and the antibody against the LII subunit was expected to be strong, but it was observed to be weak. We do not know if the structures of conglutin γ from the 2 lupin species, *L. albus* and *L. mutabilis*, used here are different

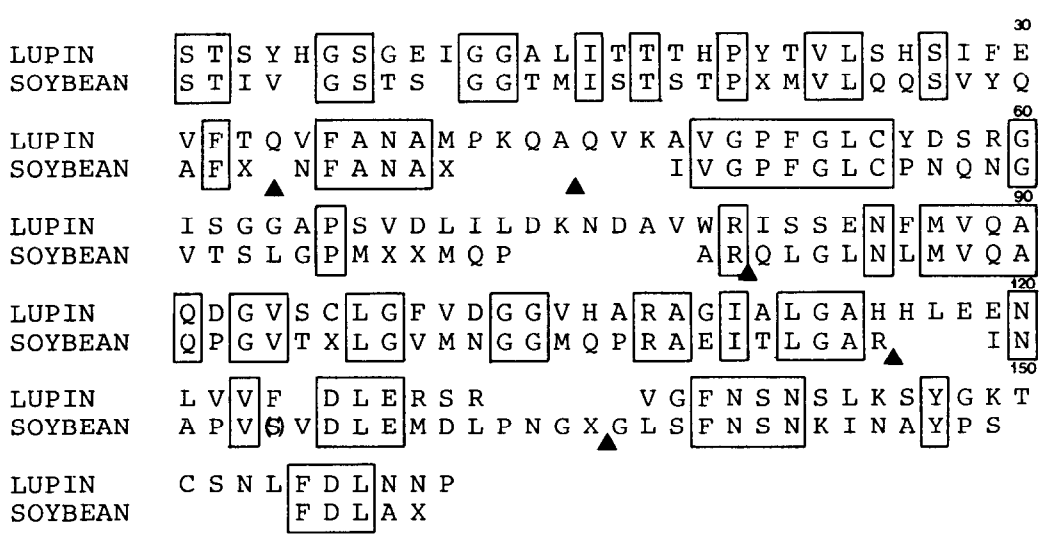

Fig.1. Structural homology between the amino acid sequences of the lupin conglutin γ and the soybean basic 7 S globulin LII subunit. Identical residues are enclosed in boxes. Triangles indicate that overlapping peptides were not available in these regions of the LII subunit.

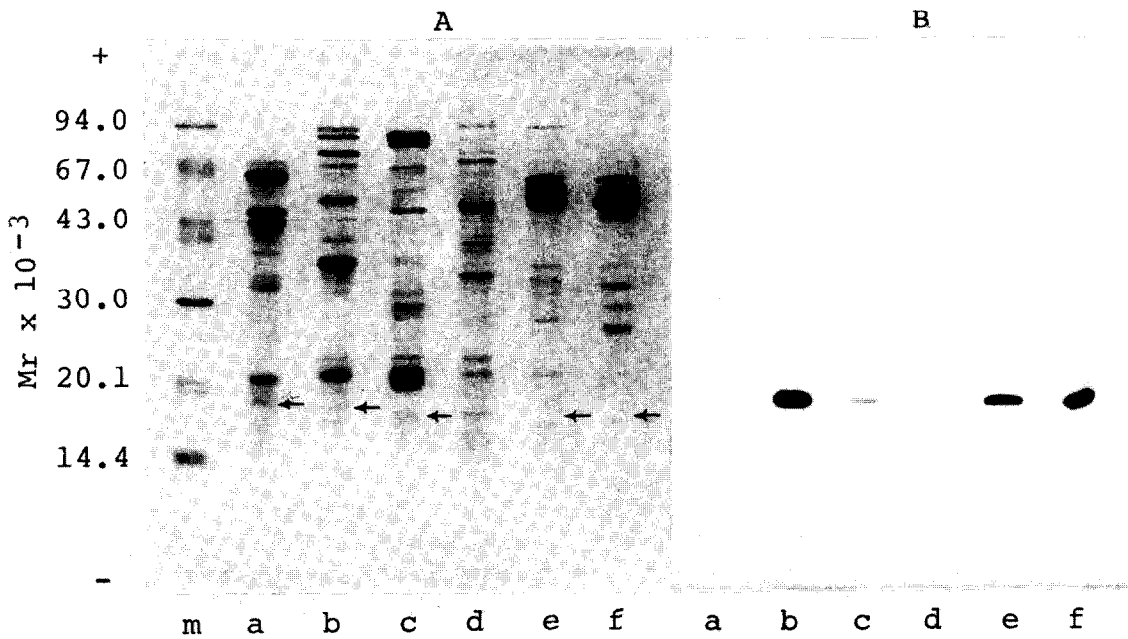

Fig.2. Analysis of legume seed proteins by SDS-polyacrylamide gel electrophoresis. (A) Total seed proteins shown by Coomassie blue staining; (B) polypeptides reacting with antiserum to the LII subunit after Western blotting of the gel shown in A. Lanes: a, *L. albus*; b, *G. max*; c, *P. tetragonolobus*; d, *P. sativum*; e, *V. angularis*; f, *V. radiata*. Lanes A and Bb, extracts of 16 μg endosperms were electrophoresed; in others, 100 μg. Lane m, molecular mass marker proteins.

from that of *L. angustifolius* (published; [5]). The weak cross-reactivity of the antibody against the LII subunit to the conglutin γ, as observed here, may be due to the structural differences in the conglutin γ among lupin species.

Additionally, it should be noted that all of the proteins that cross-reacted with the antiserum against the LII subunit in the legume species have almost the same molecular mass as the LII subunit (see fig.2A).

The results obtained here suggest that proteins similar to the soybean basic 7 S globulin are widely distributed in legume species. Previously, it was considered that only two types of major seed storage proteins, legumin- and vicilin-like proteins, were distributed in legume species. The basic 7 S globulin-like protein may be the third type of major storage protein commonly observed in many legume species.

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