Characterization of Buckwheat Seed Storage Proteins

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The major storage protein of buckwheat seed is the 13S globulin. Separation of buckwheat seed proteins by sucrose density gradient revealed the existence of an additional new minor storage protein. Analysis of 13S and the new minor class storage proteins by two systems of two-dimensional gel electrophoresis showed that the 13S globulin resembles a structure of legumin-like seed storage proteins, but the new protein appears to be a vicilin-like storage protein. The 57−58 kDa polypeptides, previously described as the unusual subunits of the 13S storage protein, in fact are the subunits of the minor class of buckwheat seed storage proteins. The major and minor classes of storage protein represent about 33 and 6.5% of total seed proteins, respectively.

Keywords: *Fagopyrum esculentum Moench; buckwheat; seed storage protein*

INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Moench) belongs to the family Polygonaceae. Chemical analysis of buckwheat proteins has shown that the amino acid composition is nutritionally superior to those of cereals and indicated that buckwheat was the best known source of high biological value proteins in the plant kingdom (Pomerantz and Robbins, 1972).

The buckwheat storage proteins have not been fully characterized. The main components of buckwheat seed proteins are salt-soluble globulins (43% of total seed proteins), represented mainly by the 13S protein fraction with M_r 280 000 (Belozersky, 1975). It has been classified as a legumin-like storage protein (Derbyshire et al., 1976) mainly according to its sedimentation constant and predicted subunit assembly in mature protein (Belozersky and Dunaevsky, 1983). Little information is available on its structure compared to that on legume or cereal storage proteins (Casey et al., 1986).

In this study, we have fractionated the total buckwheat seed globulins on linear sucrose density gradient and analyzed the structure of protein fractions by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and isoelectric focusing (IEF).

MATERIALS AND METHODS

Plant Material. Buckwheat (*F. esculentum* Moench cv. Daria) was field-grown at the Institute of Biological Research in Belgrade. The mature whole seeds were harvested and used immediately or stored in liquid nitrogen for protein extraction.

Preparation of the 31000*g* Supernatant. Seeds were ground in liquid nitrogen and extracted for 1.5 h at 20 °C in 10 volumes (w/v) of buffer containing 0.035 M potassium phosphate, pH 7.6, 0.5 M sodium chloride, and 0.02% sodium azide. The extract was centrifuged for 15 min at 31000*g* (Sorvall, RC-5B, SS-34 rotor). The proteins in the supernatant were analyzed in linear sucrose density gradient (10−30%).

Sucrose Gradient. The 31000*g* supernatant was layered onto linear sucrose density gradient (32 mL, 10−30% in buffer described above) and centrifuged for 40 h at 20 °C (Beckman, L5-65, SW 28 rotor). Fractions of 1−1.2 mL were taken and proteins photometrically monitored at 280 and 260 nm. Fractions were pooled: 5−8 for peak I; 12−14 for peak II; and 19−21 for peak III. Proteins concentration was determined according to the method of Lowry et al. (1951). Proteins in the peak fractions were analyzed by two systems of two-dimensional gel electrophoresis.

Gel Electrophoresis Systems. For the first gel system, the first dimension was run on a 0.75 mm thick 12.5% acrylamide slab gel, 12 × 14 cm, in the absence of β-mercaptoethanol (Laemmli, 1970). Gel was stained with Coomassie brilliant blue to visualize polypeptides. A strip was excised and incubated in sample buffer (50 mM Tris-HCl, pH 6.8, 2% SDS) containing 4% β-mercaptoethanol to reduce protein subunits and placed in the slot on the second dimension (12.5% acrylamide gel, 12 × 14 cm, 1.5 mm thick). The electrophoresis was run at a constant current of 20 mA per gel.

In the second two-dimensional gel system, the first dimension (IEF) was performed in a 4% acrylamide gel containing 0.4% ampholines, pH range 3−10 (O'Farrell, 1975). Proteins in the 31000*g* supernatant were loaded on the basic end of the tube gels and focused for 18 h at 400 V. The second dimension was the same as in the first system.

RESULTS

The sedimentation profile of the protein in the 31000*g* supernatant revealed the presence of three protein fractions (Figure 1A). The determination of protein concentrations in pooled peak fractions shows that fractions I, II, and III account for 75, 15, and 10% of proteins, respectively. According to SDS−PAGE under nonreducing conditions, the major fraction, I (Figure 1B), corresponded to 13S globulin and consisted of about eight different forms of subunits within a M_r range from 43 000 to 68 000. The minor fraction, fraction II, consisted mainly of 57 000−58 000 polypeptides and a few polypeptides in the range 26 000−36 000.

Separation of the 13S globulin under reducing conditions (+β-mercaptoethanol) produced two polypeptide groups: a larger group, from 32 000 to 43 000, and a smaller group, from 23 000 to 25 000 (Figure 2B). Figure 3 illustrates the differences in isoelectric points between larger and smaller polypeptides of 13S globulin. The large polypeptides were focused in the acidic region of the gel, while a part of the small polypeptide group was focused in the region of about pH 6.8. A complete set of basic polypeptides was not detected by IEF because the pH range was from 4.3 to 7.7.

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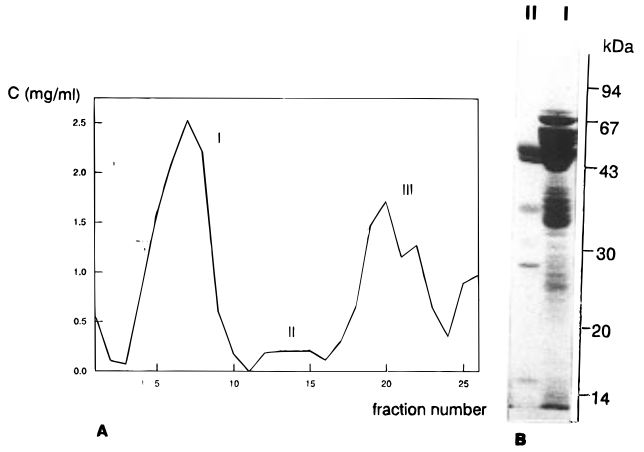

Figure 1. (A) Sucrose gradient profile of the 31000*g* supernatant. Protein concentration is expressed as $1.55A_{280} - 0.76A_{260}$. (B) Fractions were collected and peak fractions (I, II, III) analyzed on 12.5% SDS−PAGE under nonreducing conditions. Molecular weight markers are indicated on the right.

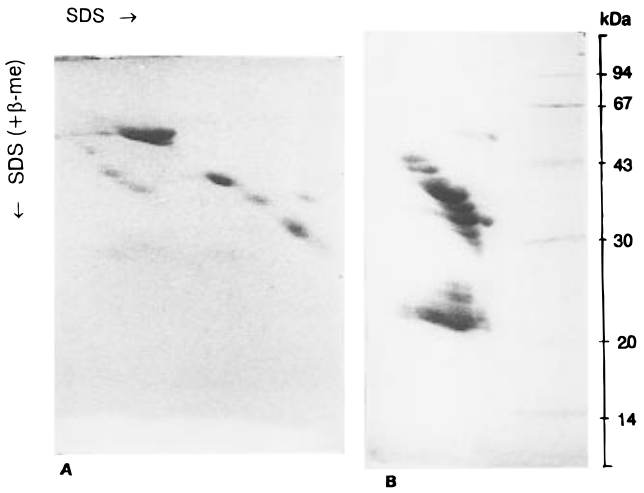

Figure 2. Two-dimensional nonreducing−reducing SDS−PAGE pattern of (A) the minor class of buckwheat storage protein and (B) the 13S buckwheat protein. Molecular weight markers are indicated on the right.

The 57 000−58 000 polypeptides, constituents of the minor class of buckwheat globulins, remained unchanged under reducing conditions (Figure 2A) and had isoelectric points of about 6.8 (Figure 3).

Fraction III consisted of polypeptides within the M_r range of 14 000−23 000. The polypeptides of 14 000−16 000 were predominant and remained unchanged under reduction (not shown).

DISCUSSION

Fractionation of buckwheat seed globulins on sucrose density gradient confirmed that 13S globulin was a major component of the buckwheat storage proteins. It also revealed the existence of new minor class storage proteins that had not been previously detected by gel filtration separation (Belozersky and Dunaevsky, 1983).

These two protein fractions had different distributions in the seed. The minor class resided both in endosperm and in cotyledons, while the 13S protein was found only in cotyledons (not shown).

Analyzed by two systems of two-dimensional gel electrophoresis, the main fraction behaved as legumin

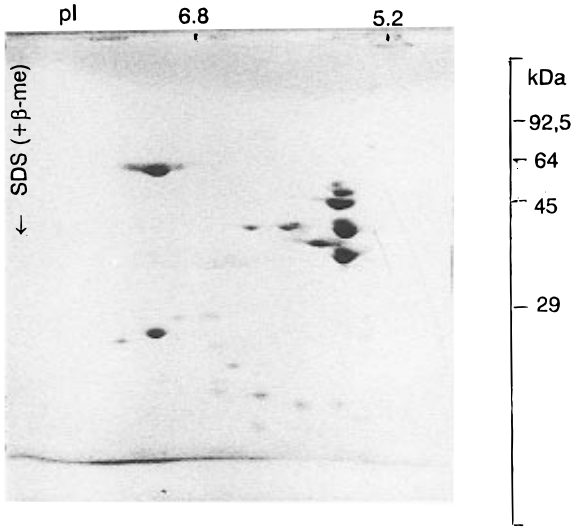

Figure 3. Two-dimensional gel pattern of buckwheat globulins. The first dimension was equilibrium IEF. The second dimension was reducing SDS−PAGE. Molecular weight markers are indicated on the right. Isoelectric points are indicated at the top of the gel.

type proteins, composed of nonidentical subunits consisting of one acidic and one basic polypeptide linked by disulfide bonds (Figure 2B). Heterogeneity was observed in both classes of polypeptides, but it was more prominent in the acidic group (at least 10 acidic and 5 basic polypeptides were detected).

Analysis of the newly detected minor storage protein class has shown that it was composed of subunits with M_r of about 57 000−58 000, which remained unchanged after reduction (Figures 1B and 2). The 57 500 polypeptide was assigned to 13S globulin (Dunaevsky and Belozersky, 1989b). Coelution of this protein with 13S globulin was probably due to its aggregation, as has been observed for some vicilins (Derbyshire et al., 1976). Its sedimentation constant of about 8 S, estimated by comparison with the corresponding sucrose gradient (Spielmann et al., 1982), as well as the time course of its biosynthesis throughout seed development (Maksimovic et al., 1995) also suggests that it could be classified as a vicilin-like protein, not described until now in buckwheat seeds.

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LITERATURE CITED

Belozersky, M. A. Isolation and characterization of buckwheat seed 13S globulin. In *Biosynthesis of Storage Proteins*; Nauka: Moscow, 1975; pp 152−156.

Belozersky, M. A.; Dunaevsky, Y. E. Initial changes of the main storage protein in germinating buckwheat seed. *Biochimiya* **1983**, *48*, 508−511.

Belozersky, M. A.; Dunaevsky, Y. E.; Elpidina, E. N. Sequential participation of proteases in hydrolysis of the main storage protein in buckwheat seeds. *Bioorg. Khim.* **1994**, *20*, 242−248.

Casey, R.; Domoney, C.; Elis, N. Legume storage proteins and their genes. *Oxford Surv. Plant Mol. Cell. Biol.* **1986**, *3*, 1−95.

Derbyshire, E.; Wright, D. J.; Boulter, D. Legumin and vicilin, storage proteins of legume seeds. *Phytochemistry* **1976**, *15*, 3−24.

Dunaevsky, Y. E.; Belozersky, M. A. The role of cysteine and carboxypeptidase in the breakdown of storage proteins in buckwheat seeds. *Planta* **1989a**, *179*, 316−322.

Dunaevsky, Y. E.; Belozersky, M. A. Proteolysis of the main storage protein of buckwheat seeds at the early stage of germination. *Physiol. Plant.* **1989b**, *75*, 424−428.

Dunaevsky, Y. E.; Belozersky, M. A.; Elpidina, E. N. Proteolytic enzyme from buckwheat seeds hydrolysing the main storage protein of the seed. *Biokhimiya* **1983**, *48*, 572−576.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.

Lowry, O. M.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

Maksimovic, R. V.; Varkonji-Gasic, I. E.; Radovic, R. S.; Savic, P. A. The biosynthesis of 13S buckwheat seed storage protein. *J. Plant Physiol.* **1995**, submitted for publication.

O'Farrell, P. H. High-resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **1975**, *250*, 4007−4021.

Pomeranz, Y.; Robbins, G. S. Amino acid composition of buckwheat. *J. Agric. Food Chem.* **1972**, *20*, 270−274.

Spielmann, A.; Schürmann, P.; Stutz, E. Gel electrophoretic characterisation of protein fraction soybean during seed development. *Plant Sci. Lett.* **1982**, *24*, 137−145.

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