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AMINO ACID SEQUENCE OF THE BASIC SUBUNIT OF 13S GLOBULIN OF BUCKWHEAT

MANOJ K. ROUT, NIKHIL K. CHRUNGOO* and K. S. RAO†

Department of Botany, N E Hill University, Shillong-793022, India, † Department of Biochemistry, Indian Institute of Science, Bangalore-560 016, India

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Key Word Index—*Fagopyrum esculentum*; Polygonaceae; buckwheat; basic subunit; legumin; lysine.

Abstract—A 26 kDa basic subunit of 13S globulin has been purified from grains of common buckwheat (*Fagopyrum esculentum* Moench). The amino acid composition of the protein closely matches the W.H.O. recommended values for a nutritionally balanced protein. The sequence of 17 *N*-terminal amino acid residues of the protein revealed 73.3 and 66.7% homology with soya bean glycinin and pea legumin, respectively.

INTRODUCTION

Seed storage proteins constitute an important source of dietary proteins for human consumption. Although cereals and legumes are a major component of human diet, the seed storage proteins in both are generally deficient in essential amino acids, especially lysine [1–4]. While many attempts have been made to improve the amino acid composition of seed storage proteins through conventional breeding programmes [5], molecular approaches provide alternative strategies towards this goal. An important approach towards improving the amino acid composition of seed storage proteins could be to express the gene for a heterologous protein with balanced amino acid composition. This approach is, however, constrained by scanty information with respect to suitable heterologous proteins. While a number of seed proteins rich in sulphur containing amino acids are available [4, 6], not many lysine rich seed storage proteins have been identified so far.

Common buckwheat is a pseudocereal with a high protein content (18%) in its grains [7]. The main seed storage protein in the plant, a 280 kDa globulin, has 6% lysine which is much higher than that reported for other cereals [7, 8]. The protein is composed of three size classes of polypeptides with M_r ranging between 55–60 (α), 32–44 (β) and 16–29 kD (γ). The present paper reports the purification and *N*-terminal amino acid sequence of a 26 kDa lysine rich subunit of the 13S globulin from grains of common buckwheat.

* Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

When screened by SDS-PAGE, the purified protein resolved into a single band corresponding to a M_r of 26 kDa (Fig. 1). The amino acid composition of the protein revealed high levels of lysine, threonine, leucine and sulphur containing amino acids (Table 1). The protein also has a high content of arginine and glutamine/glutamic acid. The presence of high levels of arginine is consistent with the role of crystalloid seed storage proteins as a nitrogen source during seed germination [3]. Also, the content of glutamine + glutamic acid (Glx) in the protein is amongst the highest values reported in the literature. Interestingly, the amino acid composition of the protein closely matches the W.H.O. recommended values for a nutritionally balanced protein.

The sequence of 17 *N*-terminal amino acid residues of the protein and alignment of the sequence with corresponding regions of proteins from grains of some important crops is presented in Fig. 2. Using an alignment that permitted maximum homology, the buckwheat protein showed 73.3% homology with soya bean glycinin, 66.7% homology with pea legumin and 46.7% homology with the β-subunit of 11S cucumber globulin. Of the 17 residues compared, six were conserved and five matched closely. The closely matching residues were amino acids with similar functional groups, thereby representing conservative replacements. Amongst the conserved residues were cysteine–threonine at position 7–8 and asparagine–isoleucine at position 14–15. A significant aspect revealed by the alignment was the presence of lysine and serine at position numbers 16 and 17 in the 26 kDa globulin

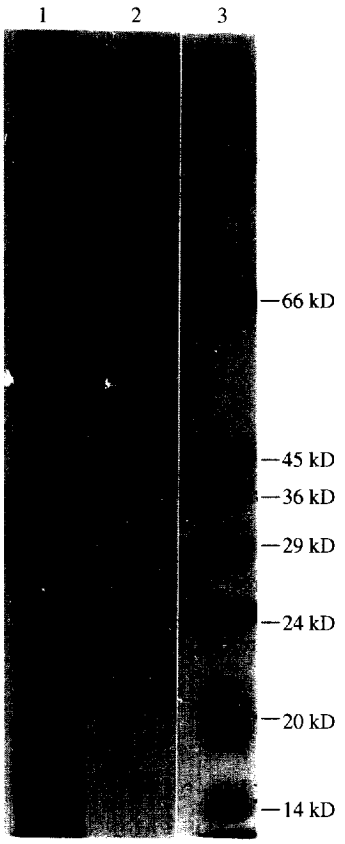

Fig. 1. SDS-PAGE of the purified 26 kDa subunit isolated from grains of common buckwheat (lane 1, 13S globulin; lane 2, purified 26 kDa subunit; lane 3, molecular weight markers).

Table 1. Amino acid composition of the purified 26 kDa basic subunit of the 13S globulin from grains of common buckwheat

Amino acid	Residues (mol %)
Asx	12.30
Glx	25.70
Ser	5.11
Thr	2.73
His	2.40
Gly	10.40
Ala	4.06
Tyr	2.13
Arg	5.93
Met	1.57
Val	3.00
Pro	2.57
Phe	1.93
Lys	6.93
Ile	3.02
Leu	6.17
Trp	*
Cys	1.03

* Not determined.

subunit: no other sequence had these residues at this position. This difference could have a correlation with phylogenetic distance between buckwheat and legumes.

Globulins from a range of flowering plants have been observed to have a conserved *N*-terminal sequence of Gly-Leu/Ile-Glu/Asp-Glu, the probability of the sequence at random being 1 in 10^6 sets of amino acids [9]. Such a sequence of amino acids is conserved in 26 kDa buckwheat globulin subunit also where it reads as Gly-Ile-Asp-Glu.

EXPERIMENTAL

Materials. Grains of common buckwheat (*Fagopyrum esculentum* Moench) var. BDS-1354 were procured from Regional station of National Bureau of Plant Genetic Resources, Shillong (India).

Protein purification. The dehulled grains were homogenized with chilled 50 mM Tris–Cl buffer (pH 8) containing 0.1 M NaCl, 5% PVP and 2 mM PMSF to get a 50% (w/v) homogenate. The homogenate was centrifuged at 10000 *g* for 15 min at 4°C. Proteins in the supernatant were precipitated with $(NH_4)_2SO_4$ (80% sat.); the ppt was dissolved in 50 mM Tris–Cl buffer (pH 8) containing 100 mM EDTA and dialysed against the same buffer for 72 hr at 4°. The dialysate was centrifuged and the 280 kDa globulin was purified from the concd supernatant by chromatography on Sepharose 6B [10]. The purified globulin was reduced with 0.1 M 2-ME in 6 M urea [11] and rechromatographed on Sephadex G-120. Frs corresponding to the peak showing the highest lysine content were pooled and rechromatographed on Sephadex G-50. The protein was eluted with Tris–Cl buffer (pH 8).

Amino acid analysis. Amino acid analysis of the protein was performed according to ref. [12].

N-terminal amino acid sequence analysis. The fr. showing the highest lysine content was analysed by SDS-PAGE on a 10% gel [13]. The protein band was transferred to PVDF membrane [14]. Detection of the protein on PVDF membrane was carried out by staining with 0.1% Coomassie Blue R-250. The segment having the protein was excised, destained with Mili-Q deionized H_2O and used directly for sequencing by Edman gas phase degradation [15] using PSQ-1 Shimadzu gas phase sequenator coupled to PTH-amino acid analyzer [14].

GeneBank database search. The FASTA programme [16] was used to search for sequences in EMBL databases using the first 17 *N*-terminal amino acid residues of purified 26 kDa protein from buckwheat grains as a query sequence.

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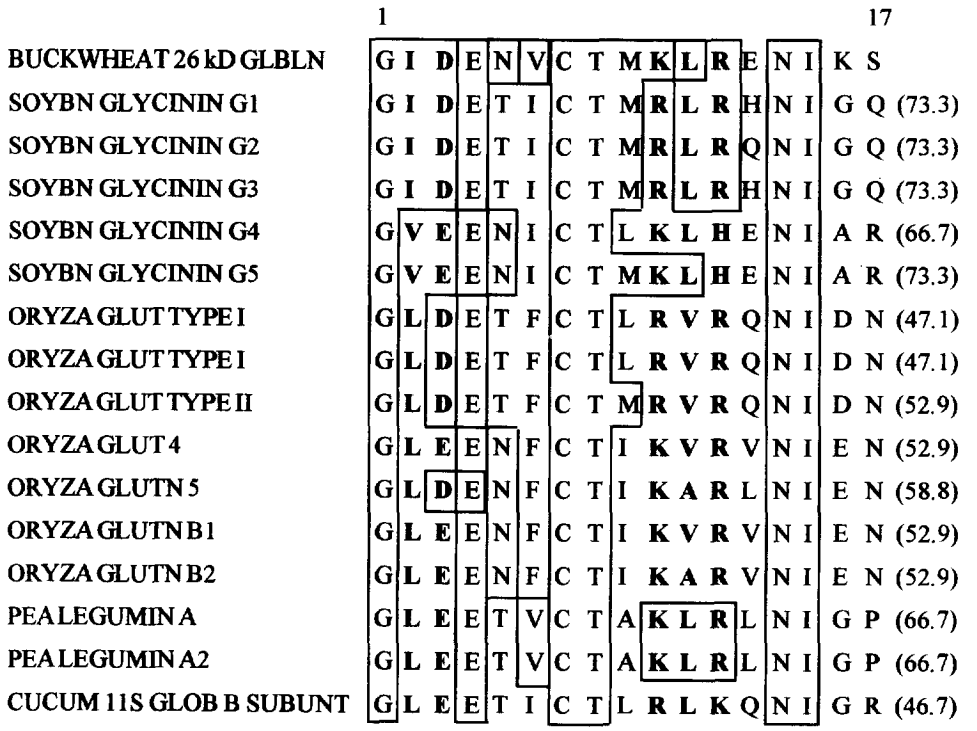

Fig. 2. Homology of the *N*-terminal region of the 26 kDa subunit of 13S globulin from buckwheat grains with the corresponding regions of soya bean glycinin [17]; pea legumin [18], β-subunit of cucumber globulin [19] and rice glutelin [20]. Conserved residues are boxed with solid lines and identical amino acids are in bold type. Figures in parentheses represent per cent homology.

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