Characterization of Vicilin Seed Storage Protein of Chickpea (Cicer arietinum L.)

A D Mandaokar, K R Koundal*, Rekha Kansal and H C Bansal
Bioctechnology Centre, Indian Agricultural Research Institute, New Delhi 110 012, India

Vicilin, one of the major storage proteins of chickpea (Cicer arietinum L.) was purified and characterized during seed development. Vicilin was purified by zonal isoelectric precipitation followed by chromatography on DEAE-cellulose column. Vicilin on SDS-PAGE resolved into 5 major bands ranging in mol wt from 14 to 66 kDa. More heterogeneous pattern emerged on isoelectric focussing. This protein had high amount of amides and low amount of sulphur containing amino acids.

Key words: chickpea, storage proteins, vicilin, isoelectric focussing

The storage proteins from legumes provide a major portion of dietary proteins for human nutrition. These proteins are salt soluble and resolve into two major fractions viz. legumin and vicilin. The proportion of these two fractions varies from species to species e.g. in Phaseolus, vicilin fraction is more than legumin while in Vicia faba legumin is found in abundance. In pea both the fractions constitute about 50-55% of the total protein. There is similarity between isoelectric point, subunit composition and amino acid content in storage proteins of various legumes. Because of high content of amides in globulins in legume seeds, it was suggested that these proteins have a storage role.

Characterization of storage proteins of chickpea is a pre-requisite for isolating genes for improving protein quality. Recent techniques have been used to bring in more refinement in the purification of storage proteins of various legumes. While considerable literature is available on changes in protein during seed development in Pisum sativum (2) Vicia faba (3) and Glycine max (4), the study of storage proteins of chickpea is scanty. Therefore, in the present study characterization of vicilin of chickpea during seed development has been done.

Materials and Methods

Plant material—Chickpea (Cicer arietinum L cv. Pusa 256) was sown in I.A.R.I. field and tagged when flowers fully opened. The pods were harvested at different stages starting from 9 days after flowering (DAF) to maturity. The seeds were removed, frozen and stored at -80°C for further analysis.

Isolation of vicilin—After removal of albumins from 20 g fresh seeds with 200 ml of McIlvaine buffer, pH 4.5 (0.043M Na$_2$HPO$_4$, 0.026M citric acid), a crude vicilin fraction was extracted with 0.2M NaCl in McIlvaine buffer pH 4.7 (0.096M Na$_2$HPO$_4$, 0.052M citric acid). Extraction was carried out for 16 h in cold. The extract was centrifuged at 10000 xg for 30 min. Supernatant containing crude vicilin was dialysed against running cold distilled water for 24 h and precipitate was recovered by centrifugation.

Purification of vicilin—Vicilin was purified by zonal precipitation (5). A Sephadex G-50 column (2.0 x 50 cm) was equilibrated with McIlvaine buffer, pH 8.0 (0.138M Na$_2$HPO$_4$, 0.071M citric acid). The sample was then dissolved and separated with McIlvaine buffer, pH 7.0 (0.165 M Na$_2$HPO$_4$, 0.018 M Citric acid) at the rate of 45 ml/h and using Frace-100 Pharmacia fraction collector. Vicilin fraction was collected and precipitated with 10% TCA. Further purification was achieved by ion-exchange chromatography on DEAE-Cellulose column (2.2 x 30 cm) equilibrated with 50 mM Tris-HCl buffer (pH 8.0). The samples were eluted with 0.1-0.4 M gradient of NaCl column buffer. Fractions were precipitated, lyophilized and analyzed by SDS-Polyacrylamide gel electrophoresis (SDS-PAGE).

Alternatively, proteins were also rechromatographed on Sepharose 4B column (2.2 x 50 cm) equilibrated with phosphate buffer, pH 8.0 (0.1M Na$_2$HPO$_4$, 0.1M NaH$_2$PO$_4$) and extract of chickpea seeds was made 85-100% saturated with respect to ammonium sulphate at 4°C. The precipitated material was collected by centrifugation at 23000xg for 20 min, dialyzed for 24 h in running distilled water and precipitated, lyophilized and then analyzed by SDS-PAGE.
**Gel electrophoresis**—Analytical gel electrophoresis was performed in 10% SDS-acrylamide gel (pH 8.3) by method of Laemmli (6). Approximately 200 μg protein was loaded on the gel and run at constant current of 10 mA for 8h. Low mol wt markers were also used for determining the mol wt of subunits. After the run, gel was stained in destaining solution (methanol : acetic acid : H₂O :: 5 : 1 : 4) containing 0.1% coomassie brilliant blue R-250 dye for 3h and destained for 4-6 h.

**Isoelectric focussing**—Isoelectric focussing of vicilin was done according to Vesterberg (7) in 5% acrylamide slab gel containing 8M urea. Focussing was carried out for 2h at 15W. After focussing, the gel was taken out and soaked in fixing solution (10% TCA + 5% sulfosalysilic acid) for 1 h. Ampholins were removed by putting the gel in destaining solution for 30 min., staining and destaining was done as described earlier.

**HPLC analysis**—Extracted vicilin was dissolved in 20mM sodium phosphate buffer (pH 6.8) and 10 μl sample was loaded on the column (weak anion exchanger, pore size 300Å). The sample was eluted with the linear gradient of 20mM to 500 mM sodium phosphate buffer (pH 8.0) at the rate of 1 ml/min and detected at 220 nm using Model LC-GA Shimadzu HPLC.

**Amino acid composition**—Samples (5 mg) were hydrolyzed in 3 ml of 6N HCl in hydrolyzing tubes and heated at 110°C for 24 h. Excess HCl was then removed by washing 5 times with Milli Q water by Flash evaporator. The dried samples were dissolved in 2.5 ml of sample diluent buffer (0.2N sodium citrate, pH 2.2) and filtered with 0.45 μM pore-sized membrane filters and used for amino acid analysis by LC-6A Shimadzu HPLC.

**Results and Discussion**

The fresh weight of the seed increased from 17 to 29 DAF and subsequently declined towards maturity. The dry weight of seeds continued to increase from 9 DAF at a slower rate and from 17 to 29 DAF at a higher rate, and then decreased towards maturity.

**Protein**—Total protein content of seed varied from 22-25% on dry weight basis. Globulin constituted about 68% of the total protein and vicilin was approximately 1/3rd of the total globulin. The pattern of vicilin accumulation showed linear increase initially (9-17 DAF) but increased sharply at later stages of seed development (21-29 DAF). Accumulation of vicilin decreased at maturity (Fig. 1). The present results in chickpea are in agreement with the results of vicilin reported by other workers (3, 8).

**Purification of vicilin**—Purified vicilin resolved as a single peak (Fig. 2). Appropriate fraction was precipitated by 10% TCA and lyophilized. On DEAE-cellulose