Phytochemistry, Vol. 24, No. 10, pp. 2201–2203, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

VICILIN FROM *CAJANUS CAJAN* SEEDS

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(*Received* 16 *January* 1985)

Key Word Index—*Cajanus cajan*; Leguminosae; pigeon pea; seed protein; vicilin.

Abstract—Vicilin from pigeon pea (*Cajanus cajan*) seeds was purified and characterised. It has a M_r of *ca* 180 000 and consists of two types of subunits having M_rs of *ca* 72 000 and 57 000. The subunits are not linked by disulphide bonds. The vicilin of pigeon pea differs from that of *Pisum* or *Vicia* in the absence of small M_r subunits.

INTRODUCTION

Pigeon pea is a tropical grain legume which is widely grown in India, Africa and parts of Latin America. It is an important source of proteins in the largely cereal based diet of the Indian population [1]. In an earlier paper we reported the presence of three fractions (α, β and γ) in the globulin protein from pigeon pea seeds [2]. The α and β-fractions correspond in their properties respectively to legumin and vicilin from other legumes. In this paper we report the purification and characterisation of vicilin from pigeon pea seeds.

RESULTS

The globulin protein elution pattern on a zonal isoelectric precipitation column is shown in Fig. 1. The first peak consisted mostly of vicilin while the retarded second peak that eluted with change in pH consisted mainly of legumin with some vicilin. The first peak when rechromatographed showed only one peak without any trace of the second peak. This protein, although homogeneous, was subjected to DEAE Sephacel ion exchange chromatography. Only one peak of protein was obtained eluting at *ca* 0.23 M NaCl.

The globulin from pigeon pea gave three bands, all with a net negative charge at pH 7.2, when electrophoresed on cellulose acetate membrane (CAM). The two fast moving fractions accounted for the bulk of the protein. The purified vicilin on the other hand gave a single band on CAM which coincided in mobility with the middle band of the globulin. In a continuous polyacrylamide gel electrophoretic system the purified vicilin gave a single band indicating the homogeneity of the protein.

The vicilin holoprotein gave a single symmetrical peak (M_r *ca* 180 000) on a Sepharose 6B column. On sucrose density gradient centrifugation the vicilin gave a single peak with a sedimentation coefficient of 7.5 s, corresponding to an M_r of *ca* 183 000 (data not shown) in good agreement with the value estimated by column chromatography, and is in good agreement with the sedimentation coefficient reported for vicilin from other legumes [3].

The electropherogram of globulin and vicilin on SDS-polyacrylamide gel is shown in Fig. 2. There were a

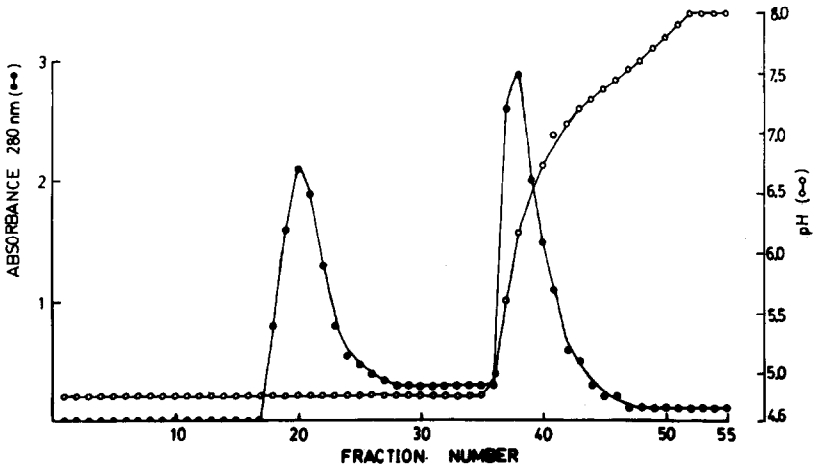

Fig. 1. Elution profile of seed globulins on a zonal isoelectric precipitation column (2.4 × 67 cm). Column was eluted at a flow rate of 55 ml/hr and 5.5 ml fractions were collected. *A* at 280 nm (●——●) and pH (○——○) was measured in the fractions.

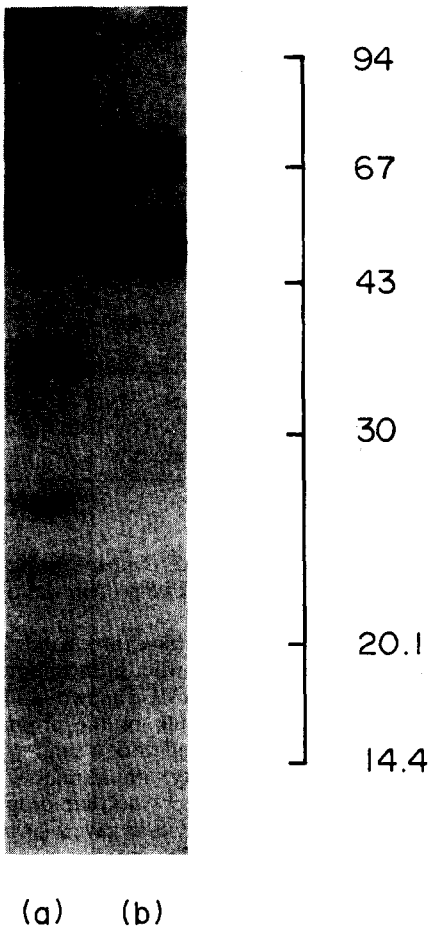

Fig. 2. SDS-polyacrylamide gel (10%) electrophoresis of (a) globulin and (b) vicilin. Protein (20 μg) was loaded in each track after treatment with SDS and 2-mercaptoethanol. The numbers at the side indicate the position occupied by marker proteins of these particular M_rs.

number of subunits in the globulin protein ranging in M_r from *ca* 72 000 to 20 000. Vicilin showed only two subunits of M_r of *ca* 72 000 and 57 000. The relative intensities of the two subunits were in the ratio of 1:2 respectively. The banding pattern did not change when 2-mercaptoethanol was included in the buffer, as found for the vicilin from other legumes.

DISCUSSION

Pisum vicilin is probably a trimer consisting of three subunits of M_r 50 000 [4]. If it is assumed that pigeon pea vicilin is also a trimer consisting of one subunit of 70 000 and two subunits of 57 000, the estimated M_r of the holoprotein would be about 184 000. This value is in very close agreement with the M_r obtained from the sedimentation coefficient value (183 000) and also from gel filtration (180 000).

Although vicilin from *Pisum* seeds shows multiple subunits on SDS gels with M_r ranging from *ca* 50 000 to 12 500, in a cell free translation system only the M_r 50 000 subunit is synthesised [4, 5]. It has been shown that *Pisum* vicilin polypeptides in the lower M_r range are produced as a result of post translational modification [6]. A similar proteolytic post translational modification has also been reported for the vicilin from *Vicia faba* [7]. These lower M_r subunits are absent in phaseolin and conglycinin, the vicilin proteins from *Phaseolus vulgaris* and *Glycine max* respectively. By vicilin cDNA comparison, it has been shown that there is considerable evolutionary divergence around the post translational processing site in *P. vulgaris* and *G. max* as compared to *Pisum* [8]. Since the smaller M_r subunits are absent in pigeon pea vicilin, it is likely that the potential processing sites in pigeon pea vicilin might resemble that of *P. vulgaris* or *G. max*.

EXPERIMENTAL

Seeds of pigeon pea (*Cajanus cajan* (L.) Mills p.) cv. T-21 were multiplied in the experimental field of this Research Centre. Chemicals were obtained from Sigma, or were of analytical grade.

Defatted meal (40 mesh sieve) prepared from mature seeds was extracted with 0.1 M borate buffer, pH 8.2 at 4° for 3 hr with constant agitation on a magnetic stirrer. The slurry was centrifuged at 10 000 *g* for 30 min. The supernatant was passed through glass wool to remove traces of low density material that floated during centrifugation. Globulin was prepared by isoelectric precipitation from the alkaline extract by overnight dialysis against 25 mM Na citrate buffer, pH 4.7. The precipitated globulin was collected by centrifugation at 12 000 *g* for 15 min and the protein pellet was dissolved in 0.15 M NaPi buffer, pH 7.2.

Vicilin was purified from globulin by a zonal isoelectric precipitation procedure [9] in a Sephadex G-50 column (2.4 × 67 cm). The first peak of protein consisting mainly of vicilin was rechromatographed to obtain pure vicilin.

Vicilin obtained after zonal isoelectric precipitation was further purified on a DEAE-Sephacel column (2.6 × 13 cm). The column was equilibrated in 15 mM NaPi buffer, pH 8. Protein in the same buffer was applied to the column. The bound protein was eluted with a linear gradient of NaCl (0–0.4 M) in 15 mM NaPi buffer, pH 8 at a flow rate of 40 ml/hr. NaCl concn was determined by conductivity measurement and protein was monitored at 280 nm in a Beckman DB-G spectrophotometer. Appropriate fractions were pooled and protein precipitated by dialysis against 25 mM Na citrate buffer, pH 4.7. The protein pellet after centrifugation was dissolved in 0.15 M NaPi buffer, pH 7.2 and used for further analyses.

Protein was estimated either by the method of ref. [10] or by the biuret method [11] using bovine serum albumin (fraction V) as the standard protein.

Electrophoresis on CAM was carried out in a Beckman microzone electrophoretic apparatus [12]. Polyacrylamide gel slabs (5%) were used for continuous electrophoresis. Both the gel and the electrode chamber contained 0.05 M NaPi buffer, pH 7.2. About 40 μg of protein was loaded. Electrophoresis was performed at a constant current of 25 mA till the marker dye had migrated to *ca* 0.5 cm from the bottom of the gel. After electrophoresis, gels were stained in 0.2% Coomassie Brilliant Blue R-250 in MeOH–HOAc–H_2O (3:1:6) and destained in the same solvent without the dye.

Polyacrylamide gel electrophoresis in the presence of 0.1% SDS (w/v) was carried out in 10% gel slabs (2 mm thick) [13]. A constant current of 50 mA was applied. Gels after electrophoresis were fixed overnight in destaining soln before staining with Coomassie Brilliant Blue R-250 as described earlier. Mobilities were measured relative to the bromophenol blue marker dye and used to obtain approximate M_rs of protein subunits [13]. Standard proteins were obtained from Pharmacia.

Gel filtration for M_r determination of the native protein was carried out on a column of Sepharose 6B (1.6 × 56 cm) in 50 mM Tris–HCl buffer, pH 8 containing 0.5 M NaCl. The column was run at a flow rate of 12 ml/hr and 1.2 ml fractions were collected and monitored at 280 nm in a Beckman DB-G spectrophotometer. The column was calibrated by determining the elution vols (V_e) of the following standard proteins: human thyroglobulin (670 000), lactate dehydrogenase (144 000), malate dehydrogenase (70 000), ovalbumin (45 000) and β-lactoglobulin (18 000). A calibration graph was obtained by plotting V_e against the log M_r of the protein.

Acknowledgements—We would like to thank Mr. S. E. Pawar for providing the T.21 seeds and Dr. R. Mitra for his helpful suggestions during the course of the investigation.

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