EXTRACTION, FRACTIONATION AND CHARACTERIZATION OF COWPEA (VIGNA UNGUICULATA) GLOBULINS

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Summary

The proteins were extracted from cowpea seeds by buffered saline solution and the globulins were separated by dialyzing the protein extract against distilled water. Fractionation of the total globulins on Sephacryl S-200 column produced two major and one minor components. The molecular weight of the major globulin components were estimated to be greater than 250 000 and 178 000 for the globulin component (a) and (b) respectively and the latter component was the predominant globulin. The two major globulin components were free from contamination of other proteins as shown by polyacrylamide gel electrophoresis. Spectrophotometry and amino acid analyses were used as tools for characterizing the globulin components.

Introduction

Increased interest in plant proteins, for feed and food, has stimulated scientists to evaluate the legume seeds as high protein crops. Among legumes, cowpea (Vigna unguiculata) is one of the protein sources in the diet of Egyptian people and also a potential protein source in Hungary.

Studies of legume proteins were initiated by Osborne and Campbell (1898) and Osborne (1924). Two globulins, legumin and vicilin, were identified according to their solubility in salt solutions of different concentrations.

In comparison to other legumes such as *Glycine max* or *Phaseolus vulgaris*, little work has been performed on the characterization of cowpea proteins. Carasco et al. (1978a) studied the cowpea globulins by ion-exchange chromatography and zone isoelectric precipitation and reported the presence of 7 S and 11 S globulin. The solubility characteristics of cowpea proteins were also investigated by Sefa-Dedeh and Stanley (1979). Khan et al. (1980) showed that cowpea seeds contain a major globulin of a molecular weight of 170 000.

As part of a programme designed to study the legume seed proteins in our departments, this paper provides information obtained on the characterization

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of cowpea globulins with respect to the number of components. The amino acid composition as well as some physicochemical properties of the major components were investigated.

Materials and methods

Air-dried cowpea seeds (Vigna unguiculata) variety black-eyed were obtained from the Experimental Farm of El-Minya Faculty of Agriculture, El-Minya University, El-Minya Egypt. The seeds were ground to fine powder with a coffee grinder, the lipids were removed by ice cold acetone and the dry defatted meal was kept in a closed container at 4 °C until used.

Extraction of proteins

The defatted meal was homogenized in 50 mM phosphate buffer, pH 7.8, containing 0.5 *M* NaCl and 10 mM mercaptoethanol at a meal to solvent ratio of 1 to 20 (W/V). The homogenate was stirred for 8 h at 4 °C and clarified by centrifugation at 15 000 rpm for 20 min. The Pellet was discarded and the clear supernatant was dialyzed at 4 °C for 72 h against several changes of distilled water. The precipitate formed during dialysis (globulins) was collected by centrifugation at 20 000 rpm for 20 min, washed twice with distilled water and freeze dried.

Gel filtration chromatography

Sephacryl S-200 column (2–5 × 92 cm) equilibrated with 0.1 *M* Tris-HCl buffer, pH 7.6, containing 0.5 *M* NaCl was used to fractionate the cowpea total globulins. Freeze dried globulins (200 mg) were dissolved in 5 ml of the equilibrating buffer, centrifuged at 15 000 rpm for 20 min to remove undissolved materials, and the clear supernatant was transferred to the column. The elution was carried out with the same buffer and fractions of 5 ml were collected at a flow rate of 20 ml/h. The fractions were analyzed for absorbance at 280 nm and by the method of Lowry et al. (1951) at 750 nm. Absorbance readings were plotted against tube number.

Molecular weight determination

The molecular weight of the globulin components was estimated according the methods described by Andrews (1965). The following proteins

were used for calibrating the Sephacryl S-200 column: myoglobin, ovalbumin, bovine serum albumin, lactate dehydrogenase, and catalase. The void volume of the column was determined using blue dextrane.

Polyacrylamide gel electrophoresis

This was carried out using gels of 4.5% acrylamide according to the procedure of Davis (1964).

Ultraviolet absorption spectra

The absorption spectra of the protein components in the eluting buffer were recorded on Unicam SP 1800 double beam recording spectrophotometer.

Amino acid analysis

The total globulins and the components separated by column chromatography were hydrolyzed in 6 *N* HCl at 110 °C for 22 h in evacuated sealed tubes. The hydrolyzates were evaporated and washed twice with distilled water. The dried hydralyzates were then made up to known volume with 10% isopropanol (v/v). The amino acid contents were determined by descending one dimensional paper chromatography using the following solvents:

1. Buffered phenol solvent (McFarren, 1951) modified by Levy and Chung (1953), phenol : 2-butanol : 0.067 *M* phosphate buffer, pH 12, (95 : 5 : 100 v/v)
2. n-Butanol : acetic acid : water (4 : 1 : 5 v/v) (Block et al. 1955)
3. n-Butanol : acetic acid : water (4 : 1 : 1 v/v) (Roland and Gross 1954).

After spraying with ninhydrin-cadmium acetate solution. The invidual amino acids were eluted with 5 ml methanol and the absorbance was measured at 500 nm for all amino acids except proline (at 510 nm). The amount of each amino acid was determined by using its standard curve.

Determination of tryptophan

The tryptophan content was determined quantitatively according to the method of Blauth et al. (1963).

Results and discussion

Extraction and fractionation of cowpea globulins

Cowpea proteins extracted with 50 mM phosphate buffer, pH 7.8, containing 0.5 *N* NaCl and 10 mM mercaptoethanol were separated into globulins and albumins by dialysis against distilled water.

The total globulins were fractionated by a Sephacryl S-200 column. The elution profile of this column is presented in Fig. 1. It is evident that cowpea globulins could be separated into two major and one minor components designated by a, b and c in the order of their elution from the column. Although both components a and b were major, the second peak contained more protein than the first one. The elution profile of the Sephacryl S-200 column shown in Fig. 1 indicated that the globulin component (a) was eluted in the void volume what shows the presence of a protein of a molecular weight greater than

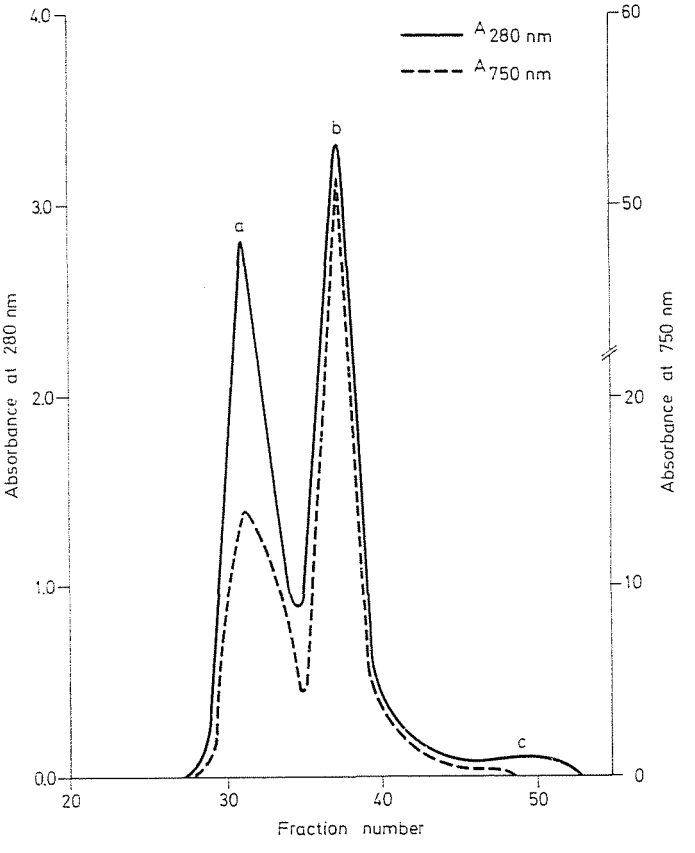

Fig. 1. Fractionation of total globulins on Sephacryl S-200 column

250 000. This component might be 115 "legumin-like" globulin mentioned by Carasco et al. (1978). A molecular weight of 300 000–400 000 was determined for this protein by gel filtration chromatography.

It has been suggested by Khan et al. (1980) that cowpea legumin contained disulphide linked pairs of subunits of molecular weight of 62 000 and 18 500.

The molecular weight of the second globulin component (peak b) was estimated by gel filtration chromatography. The value obtained was 178 000 which agreed with the results reported by other investigators (Carasco et al. 1978; Khan et al. 1980).

It has been reported by Danielsson (1949) that much of the protein of legume seeds was salt-soluble globulins and he was able to separate this fraction from *Pisum sativum* into two major components, legumin and vicilin, using repeated precipitation by dilution and heat treatment. The major globulin component (a) in our study was that with a molecular weight of 178 000. This was in a good agreement with the data reported on the protein components of globulin separated from legume seeds. They include vicilin of molecular weight of 140 000–180 000 (7 S) and legumin with molecular weight of 300 000–400 000 (11 S). The ratio of these is different in different species, for example it is 1 : 4 in *Vicia faba* (Wright and Boulter, 1972), and 9 : 1 in *Phaseolus vulgaris* (Derbyshire and Boulter, 1976). The major globulin fraction in cowpea in this study is probably 7 S globulin and this was similar to the finding of Khan et al. (1980).

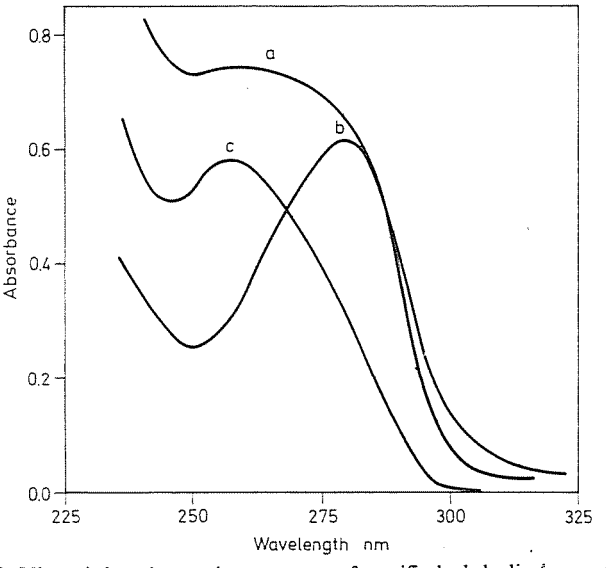

Fig. 2. Ultraviolet absorption spectra of purified globulin components

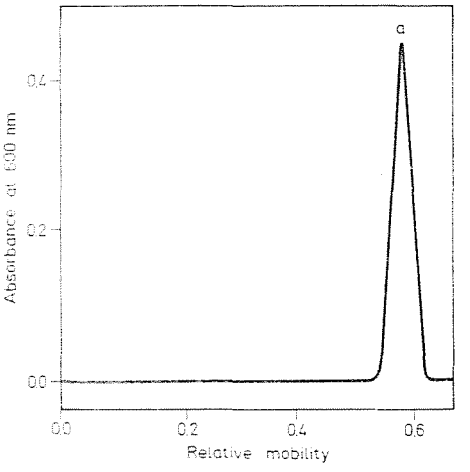

Fig. 3a. Densitometric scanning of polyacrylamide gel electrophoretic pattern of globulin component (a)

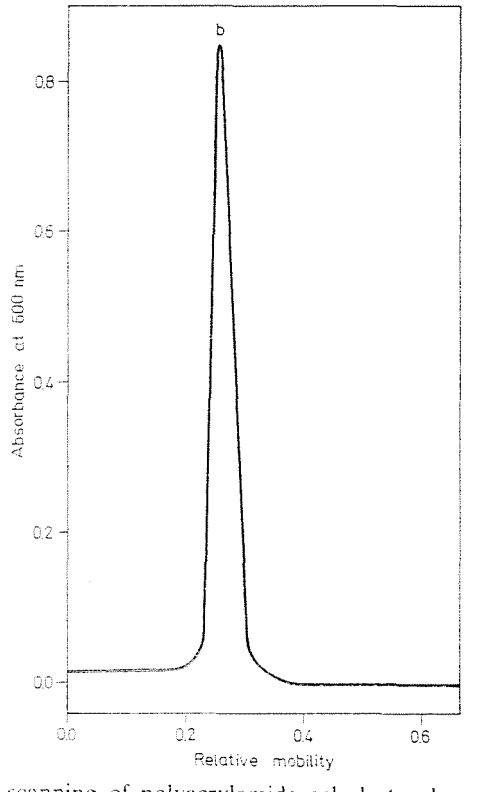

Fig. 3b. Densitometric scanning of polyacrylamide gel electrophoretic pattern of globulin component (b)

The ultraviolet absorption spectra of the globulin components obtained from the Sephacryl S-200 column in 0.1 *M* Tris-HCl buffer, pH 7.6, containing 0.5 *M* NaCl are shown in Fig. 2. The major globulin component of cowpea (peak b) in this study showed a typical protein spectrum with a maximum at 278 nm and a minimum at 250 nm. The ratio of absorbance at 280 nm to that at 260 nm, exceeded 1.55 indicating that this component was free from nucleic acid impurities (Warburg and Christian, 1942). The globulin component (a) showed a broad peak around 260 nm while the ultraviolet absorption spectrum of the minor component (c) was characterized by a maximum at 260 nm and a minimum at 248 nm. Thus it could be concluded from the ultraviolet spectrophotometric analysis that the two globulin components a and c were proteins containing nucleic acids.

The globulin components separated by Sephacryl S-200 column had to be tested for purity by polyacrylamide gel electrophoresis, since cross-contamination has been mentioned to be a problem during fractionation (Millerd, 1975). Each of the two major globulin components separated by column chromatography was free from contamination of other proteins as shown by polyacrylamide gel electrophoresis (Fig. 3a and b). The total globulins gave two bands, after staining with amido black, with relative mobility of 0.28 and 0.56 respectively. The large size globulin component (molecular weight 300 000–400 000) corresponds to the fast moving band (Fig. 3a) whereas that of molecular weight of 178 000 corresponds to the slow moving protein (Fig. 3b).

Amino acid composition

The results of amino acid analyses of the total globulins and the globulin components obtained from the Sephacryl S-200 column are presented in Table 1. In contrast to similarities in the contents of some amino acids, there were appreciable differences in the levels of other amino acids. Glutamic and aspartic acids were the predominant amino acids. They constituted about one third of the total recorded amino acids. On the other hand, tryptophan represented the lowest level of the amino acids detected.

It is evident from Table 1 that the globulin component (a) has better pattern of sulphur containing amino acids and tryptophan than the other globulin component (b) and this offers some hope that amino acid imbalance may partly be corrected by breeding and selection. The globulin component (b) was characterized by higher contents of lysine, phenylalanine, glutamic acid and alanine than the globulin component (a) and the total globulins.

Data on the amino acid composition of cowpea meal, protein concentrate and protein components were reported in the literature (Evans and Boulter,

Table 1
Amino acid composition of cowpea seed globulins (g/100 g protein)

Amino acid	Total globulins	Globulin (a)	Globulin (b)
Cystine	1.31	1.93	1.12
Lysine	5.04	5.83	7.46
Histidine	7.02	7.47	4.48
Arginine	7.13	6.83	5.69
Proline	0.66	1.03	0.75
Tyrosine	7.84	5.54	4.66
Methionine	1.86	1.22	1.12
Valine	3.07	3.09	3.36
Phenylalanine	5.07	5.57	6.81
Leucine + Isoleucine	8.64	9.02	9.00
Aspartic acid	13.89	14.12	13.31
Glutamic acid	16.45	15.96	16.73
Serine	3.51	5.54	5.32
Glycine	2.96	3.35	2.17
Threonine	5.81	3.41	4.94
Alanine	4.22	4.57	5.13
Tryptophan	0.04	0.15	0.04
Total amino acids	94.52	94.63	92.09

1974; Molina et al., 1976, Carasco et al., 1978; Khan et al., 1980; Ologhobo and Fetuge 1982). They agreed fairly well with our results for some amino acids and with reported different values for the others. These observations suggest that some similarities may exist in the amino acid composition of seed proteins between different varieties of cowpea.

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References

ANDREWS, P.: Estimation of the molecular weight of proteins by Sephadex gel filtration. Biochem. J. *96*, 595 (1965)

BLAUTH, O. J.–CHAREINSKI, M.–BERBEE, H.: A new rapid method for determining tryptophan. Anal. Biochem. *8*, 69 (1963)

BLOCK, R. J.–DURRUM, E. A.–EWEIG, G.: A manual of paper chromatography and paper electrophoresis. 2nd Ed., Academic Press Inc. Publishers, New York (1955)
CARASCO, J. F.–CROY, R.–DERBYSHIRE, E.–BOULTER, D.: The isolation and characterization of the major polypeptides of the seed globulin of cowpea (Vigna unguiculata L. walp) and their sequential synthesis in developing seeds. J. Exp. Bot. *20*, 309 (1978)
DANIELSSON, C. E.: Investigation of vicilin and legumin. Acta Chem. Scand. *3*, 41 (1949)
DAVIS, B. J.: Disc Electrophoresis. Ann. N. Y. Acad. Sci. U. S. *121*, 404 (1964)
DERBYSHIRE, E.–BOULTER, D.: Isolation of legumin-like protein from *Phaseolus aureus* and *Phaseolus vulgaris*. Phytochemistry *15*, 411 (1976)
EVANS, I. M.–BOULTER, D.: Chemical methods suitable for screening for protein content and quality in cowpea (Vigna unguiculata) meals. J. Sci. Fd. Agric. *25*, 311 (1974)
KHAN, R. I.–GATEHOUSE, J. A.–BOULTER, D.: The seed proteins of cowpea (Vigna unguiculata L. Walp.) J. Exp. Bot. *31*, 1599 (1980)
LEVY, A. L.–CHUNG, D.: The dimensional chromatography of amino acids on buffered papers. Anal. Chem. *25*, 396 (1953)
LOWRY, O. J.–ROSENBROUGH, N. J.–PARR, A. L.–RANDALL, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. *193*, 265 (1951)
MCFARREN, V. F.: Buffered filter paper chromatography of the amino acids. Anal. Chem. *25*, 168 (1951)
MILLERD, A.: Biochemistry of legume seed proteins. Ann. Rev. Plant Physiol. *26*, 53 (1975)
MOLINA, M. R.–ARGUETA, C. E.–BRESSANI, R.: Protein-starch extraction and nutritive value of the black-eyed pea (Vigna sinensis) and its protein concentrates. J. Food Sci. *41*, 928 (1976)
OLOGHOBO, A. D.–FTUGA, B. L.: Chemical composition of promising cowpea (Vigna unguiculata) varieties. Nutr. Rep. Inter. *25*, 913 (1982)
OSBORNE, T. B.: "The vegetable proteins" Longmans, Green and Co., London (1924)
OSBORNE, T. B.–CAMPBELL, G. F.: Proteins of the pea. J. Am. Chem. Soc. *20*, 348 (1898)
ROLAND, J. F.–GROSS, A. M.: Amino acid analysis of Beta-Lactoglobulin by paper chromatography method. Anal. Chem. *26*, 502 (1954)
SEFA-DEDEH, S.–STANLEY, D.: Cowpea proteins. 1. Use of response surface methodology in predicting cowpea (Vigna unguiculata) protein extractability. J. Agric. Food Chem. *27*, 1238 (1979)
WARBURG, O.–CHRISTIAN, W.: Isolation and crystallization of enolase. Biochem. Z. *310*, 384 (1942)
WRIGHT, D. J.–BOULTER, D.: Characterization of vicilin during seed development in *Vicia faba*. Planta *105*, 60 (1972)

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