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GLOBULIN STORAGE PROTEINS OF *GLYCINE MAX*

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Abstract—Disc electrophoresis of soybean storage globulins indicated four major components with molecular weights in excess of 300 000. Treatment with sodium dodecylsulphate (SDS) resulted in an increased number of components with an overall lower MW spectrum. Polypeptides, produced by the performic acid oxidation of the globulins, gave 8 bands when solubilized in 2·5% NaCl, but only 4 when solubilized in 0·1% SDS. Reasons for this reduction of subunit number are suggested. Preparative fractionation of the SDS-polypeptides on Sephadex G75 columns gave the same number of components, having a similar distribution and proportion.

INTRODUCTION

THE MAJOR storage protein of soybean (*Glycine max*) is a globulin traditionally called 'glycinin'.[1] There is ample evidence that this protein is heterogeneous.[1] Sedimentation studies have shown that the globulins can be dissociated into smaller units by treatment with certain salts[2] and/or thiols.[3] Other workers have stated that subunits of glycinin have MWs as low as 20 000[4,5] and aggregates MWs as high as 363 000,[6] in agreement with the present findings.

In addition to its intrinsic interest, the present study is a prerequisite to methods of improving the quality of plant seed proteins, which is primarily determined by the balance of certain essential amino acids.[7]

RESULTS

Disc electrophoresis of globulins of the soybean variety 'Merit' indicated six components, increasing quantitatively with increasing electrophoretic mobility (Fig. 1a). The fastest running component comprises as much as 70% of the total on the basis of the densitometer tracing of the stained gel. The components would not penetrate normal gel concentrations (7·5%), but were readily mobile on 5% gels, suggesting MWs in excess of 300 000.[8] When these globulin components were complexed with sodium dodecyl sulphate (SDS), at least nine bands were visible (Fig. 1b) of greater overall mobility than the untreated globulins.

The constituent polypeptides of these globulins, produced by oxidation with performic acid, were electrophoresed on 7·5% gels. Eight bands were visible (Fig. 1c), in two groups of four. When treated with SDS, only four bands were obtained, the slowest running band

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[2] RACKIS, J. J., SMITH, A. K., BABCOCK, G. E. and SASAME, H. A. (1957) *J. Am. Chem. Soc.* **79**, 4655.
[3] WOLF, W. J. and SMITH, A. K. (1961) *Food Technol.* **15**, 12.
[4] KRETOVICH, V. L., SMIRNOVA, T. I. and FRENKEL, IA. S. (1959) *Biochemistry* (*U.S.S.R.*) (*English Transl.*) **23**, 547.
[5] KRETOVICH, V. L., SMIRNOVA, T. I. and KARYAKIN, A. V. (1961) *Compounds of High Molecular Weight* (vysokmolekul) **3**, 1389.
[6] WOLF, W J., BABCOCK, G. E. and SMITH, A. K. (1962) *Arch. Biochem. Biophys.* **99**, 265.
[7] ALTSCHUL, A. M. (1965) *Proteins: Their Chemistry and Politics*, Chapman & Hall, London.
[8] ANON, *Operating Instructions, Shandon Disc Electrophoresis Apparatus*, p. 3, Shandon Press, London.

being the most intensely stained (Fig. 1d). The four basic patterns (Figs. 1a–d) were found to hold for two other American soybean varieties, 'Beeson' and 'Amsoy', and also an unknown Canadian variety. When the polypeptides obtained from a commercially heat-treated sample of this Canadian variety were electrophoresed, dissolved in 2·5% NaCl, only four bands were obtained (Fig. 1e), a result similar to the SDS-polypeptide pattern (Fig. 1d), differing only in having a slightly lower overall relative mobility.

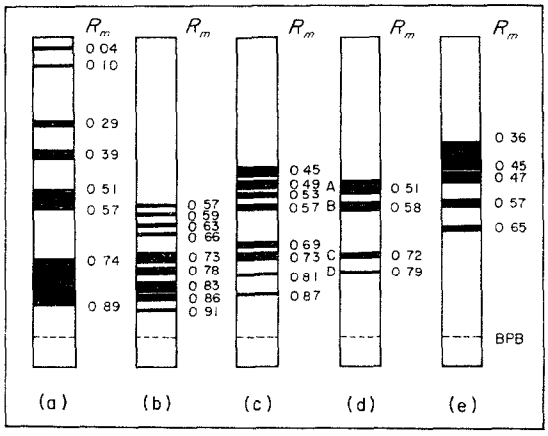

FIG. 1. ACRYLAMIDE GEL ELECTROPHORESIS OF (a) SOYBEAN CV. 'Merit' GLOBULINS IN 2·5% NaCl ON 5% GEL, (b) 'Merit' GLOBULINS IN SDS ON 5% GEL, (c) 'Merit' POLYPEPTIDES IN 2 5% NaCl ON 7·5% GEL, (d) 'Merit POLYPEPTIDES IN SDS ON 7 5% GEL (e) POLYPEPTIDES IN 2·5% NaCl ON 7·5% GEL: HEAT-TREATED UNKNOWN CANADIAN VARIETY. BPB = BROMOPHENOL BLUE MARKER BAND. Origin at top.

Preparative gel electrophoresis was unsuccessful because eluant flow-rates giving acceptable resolution of band components resulted in excessive dilution. When the SDS polypeptides were run on Sephadex G75 columns, the same number of peaks were found in the same relative positions and order as were the bands found on the gels. The approximate

TABLE 1. PROPORTIONS AND MWs OF SOYBEAN POLYPEPTIDES SEPARATED BY GEL-FILTRATION ON SEPHADEX G75

Component peak	Approximate relative proportion* (%)	Approximate MW	Component peak	Approximate relative proportion* (%)	Approximate MW
A	55	24 000	C	14	15 300
B	27	18 600	D	4	13 700

* Calculated as areas under peaks from absorbance at 254 nm.

proportion of each peak was estimated, together with the approximate MW (see Table 2), estimated from a calibration of the column with known MW markers. Fractions representing each peak were eluted from the column, pooled and lyophilized. Samples were redissolved and electrophoresed. With a small amount of 'overlap', peak fractions gave bands corresponding to those obtained by direct electrophoresis of the SDS-polypeptides.

TABLE 2. RATIO OF MWs* OF THE EIGHT NaCl-RUN POLYPEPTIDE COMPONENTS

Bands compared (mobilities)	MW ratio	Bands compared (mobilities)	MW ratio
0·45/0·69	2·2:1	0·53/0·81	2·2:1
0·49/0·73	2·1:1	0·57/0·87	2·2:1

* MW ratios were calculated assuming that separation due to charge difference was zero.

DISCUSSION

'Glycinin' in 2·5% NaCl when run on 5% gels gave a pattern of six bands, each component being in greater abundance with increasing mobility, suggestive of an homologous polymeric series, e.g. as in bovine serum albumin.[9] However, treatment with 0·1% SDS increased the number of components to nine, indicating that at least some of the aggregates consist of heterologous subunits; at the same time there is no significant reduction in the MW of the most mobile component.

The polypeptides show distinctive patterns when they are electrophoresed after treatment with 2·5% NaCl or 0·1% SDS. With NaCl, eight bands are observed, in two groups of four. With SDS, only four bands are formed. The slower-running group in the NaCl situation may represent dimers of the faster-running group, which SDS dissociates to four ultimate polypeptides. Since SDS treatment results in separation on the basis of MW alone,[10] an alternative hypothesis is that the eight components seen on NaCl-gels fall into 4 MW pairs, the components of each pair being of different charge, which have the same mobilities on SDS gels. When the rough visual estimates of the band intensity of the pairs of components are added together appropriately, a better fit is obtained by assuming the NaCl charge-splitting hypothesis rather than the dimer hypothesis. On the other hand, assuming that in NaCl the differential separation due to charge is small, the mobilities of the two sets of components will be related approximately to the log of the MW, as is the case in SDS gels. The ratios found (Table 2) support the dimer hypothesis, as does the direct evidence that after heat treatment of the seeds, the NaCl-run polypeptides show only four bands.

The results of the electrophoresis of Sephadex-separated SDS-polypeptides indicate that gel filtration provides a preparative-scale separation corresponding to analytical separation on SDS-gels.

Evidence suggests that there are at least four polypeptide components, unless there has been a specific cleavage of larger polypeptide(s). The determined MW of the predominant polypeptide(s) was *ca.* 24 000, which compares quite well with figures quoted elsewhere for the molecular weight of the ultimate subunit(s) of glycinin.[4,5] The values for MW of polypeptides derived from the Sephadex column calibration seem to rule out a polymeric series.

EXPERIMENTAL

Plant materials. Seed samples of *Glycine max* (cv. 'Merit', 'Beeson' and 'Amsoy') were kindly supplied by Northrup, King and Co., P.O. Box 959, Minneapolis, MN 55440, U.S.A. Heat-treated and untreated seed of an unknown Canadian variety was supplied by Soya Foods Ltd., Colonial House, Mincing Lane, London, E.C.3.

[9] DUNKER, A. K. and RUECKERT, R. R. (1969) *J. Biol. Chem.* **244**, 5074.
[10] NEVILLE, D. M., JR. (1971) *J. Biol. Chem.* **246**, 6328.

Globulin extraction. Seeds were ground in a Braun Blender and defatted with hexane until the filtrate was colourless. The meal was finally washed with Et_2O and allowed to dry in air. Defatted meal was extracted overnight with 7% KCl in 0·1 M phosphate buffer, pH 7 0, using a magnetic stirrer. The slurry was allowed to settle, and the supernatant decanted into 50 ml centrifuge tubes and spun at 3000 *g* for 30 min. Supernatants were pooled, placed in dialysis sacs (25 mm wide) and dialysed overnight against running tap water. Retentates were pooled and the precipitated globulins spun down at 3000 *g* for 10 min. Supernatant albumins were discarded, and the globulins washed at least 3 × with dist. H_2O. Precipitates were pooled, suspended in a minimum vol. of dist. H_2O and lyophilized.

Performic oxidation. 500 mg of lyophilized globulins were dissolved in 10 ml HCOOH (98%)/MeOH (4:1) and treated (at −5°) with performic acid reagent, prepared 2 hr previously by adding 1 ml 30% H_2O_2 to 19 ml 98% formic acid, and leaving at room temp. After 2 hr at −5°, the globulin/performic acid reaction was terminated by the addition of 200 ml of dist. H_2O at 0°, and the solution at once lyophilized.

Disc electrophoresis. Disc electrophoresis was carried out as described by Ornstein and Davis,[11] with certain modifications. Globulins and polypeptides were dissolved in 2 5% NaCl, made viscous with 20% sucrose and vols containing about 200 μg applied to the top of each gel. Globulins were usually run on 5% small pore gel, the large pore layer being omitted. Polypeptides were run on 7 5% small pore gels, with the usual large pore stacking gel. Polypeptides and globulins were complexed with SDS by allowing them to react with 0 1% SDS for 1 hr. The addition of SDS to the gel solns was not found to change any patterns obtained, and was routinely omitted. After removal from their running tubes, the gels were stained for 1–1 5 hr in naphthalene blue–black in 7% HOAc, and destained by successive washing in 7% HOAc.

Gel filtration. Apart from trial separations on Sephadex G200 and G100, most separations were carried out on a column 50–100 cm long (i.d. 2 5 cm), containing Sephadex G75 Samples of about 2 ml were applied (containing about 50 mg protein in SDS), and the column eluted with 0·1 M Tris-HCl, pH 8·0. Fractions of about 10 ml (150 drops) were collected and continuously monitored for absorption at 254 nm. In order to determine approximate MWs, the column was calibrated with 20 mg/ml samples of known MW markers (e.g. α-chymotrypsin, α-lactalbumin, cytochrome-*c*, pretreated for 1 hr with 1% SDS) The approximate MWs of the soybean polypeptide components was determined by reference to a plot of elution vol vs. log MW.

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[11] ORNSTEIN, L. and DAVIS, B. J. (1961) *Disc Electrophoresis*, Pre-print by Distillation Products Industries, New York.