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Polyacrylamide gel electrophoresis of soybean seed proteins

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Abstract

Four major and 14 minor protein bands were detected when total salt soluble proteins of soybean (*Glycine max* cultivar Warszawska) seed were subjected to polyacrylamide gel electrophoresis under nondissociating conditions, and 16 protein bands were detected under dissociating conditions. Molecular weights of three major protein fractions in PAGE SDS were determined for around 18 500, 36 000 and 80 000 daltons.

INTRODUCTION

Two major storage protein fractions of legume seeds, legumins and vicillins (Osborne, 1924), are now referred to most commonly by sedimentation coefficients, 11S and 7S, respectively (Derbyshire et al., 1976). Minor fractions of smaller and larger globulins, 2-4S and 15-18S, are also found in the protein extracts (*cf.* Kulka and Grzesiuk, 1978). The latter ones seem to be products of association during purification, as several legume seed globulins are known to undergo either salt- or pH-dependent reversible polymerization (Catsimpoolas et al., 1969; Koshiyama, 1968; Roberts and Briggs, 1965; Stockman et al., 1976; Wolf, 1970).

Salt extractable protein of soybean seed had been fractionated into three classes: 11.8S (glycinin), 7.5S and 2.8S, by sucrose density gradient centrifugation at high ionic strength ($I=0.5$). The 7.5S protein peak considerably diminished at low ionic strength ($I=0.1$) with simultaneous formation of a 9S protein. Only the 11.8S protein behaved as a homogenous fraction when it had been subjected to polyacrylamide gel electrophoresis (PAGE) under nondissociating conditions, whereas the others were found to be heterogenous (Hill and Breidenbach, 1974).

Molecular weight (MW) for glycinin and 7S globulins of soybean have been reported to be in a range of 309 000-380 000 and 105 000-330 000, respectively (*cf.* Derbyshire et al., 1976). The glycinin molecule seems to be built up of 12 subunits with equimolar amounts of acidic (MW 37 000) and basic (MW 22 000) ones (Catsimpoolas et al., 1967; Catsimpoolas 1969); both acidic and basic subunits were fractionated into several bands by techniques of isoelectric focussing and PAGE (*cf.* Derbyshire et al., 1976). Different molecular weights have been assigned to subunits of the 7S globulin of soybean, the values differing as much as 23 000 and 35 000 as determined by sedimentation analyses, and 23 000, 51 000 and 81 000 as determined by PAGE of urea dissociated preparations (*cf.* Derbyshire et al., 1976). There is no doubt that such large discrepancies were due to different procedures of purification resulting in the non-identity of the final protein preparations (*cf.* Kulka and Grzesiuk, 1978).

The aim of this study was to test how many bands could be detected in extracts of total soybean seed proteins subjected to electrophoresis under dissociating conditions (PAGE SDS).

MATERIAL AND METHODS

Protein extraction

Seeds of *Glycine max* Merr. cultivar Warszawska were obtained from IHAR, Radzików nr. Warsaw. The seeds were harvested in 1976, and used for analyses in spring 1977. Vigour of the seeds as measured by electroconductivity of steep water and germinability at low temperature (Knypl, 1978) was rather low. Maximum germination at 25 °C and 10 °C was around 70% and 35%, respectively.

Dehulled seeds were cracked in a Waring blendor and milled to a fine powder. Lipids were discarded with *n*-hexane (16 ml per g of meal) for 16 h at room temperature. Protein was extracted twice with 0.02 M phosphate buffer, pH 7.4, containing 2.5% (w/v) of NaCl and 0.01 M 2-mercaptoethanol (Sabir et al., 1973). Each extraction was conducted at room temperature for 45 min at a meal to solvent ratio of 1 : 10 (w/v) and continuous shaking. After clearing by centrifugation at 4 500 g for 20 min, the supernatant was subjected to gel chromatography.

Gel chromatography

The salt soluble proteins (150-180 mg) were fractionated at 6 °C on a 2.6 × 90 cm column K26/100 (Pharmacia, Uppsala) packed with Sephadex G-200. The proteins were eluted by downward flow of 2.5%

(w/v) NaCl solution in 0.02 M phosphate buffer, pH 7.4, containing 0.01 M 2-mercaptoethanol and 0.02% sodium azide as a bacteriostatic agent. Five ml fractions were collected each 20 min. For the estimation of MW, the column was calibrated by determining the elution volumes (V_e) of standard proteins. The regression between V_e/V_o and the log of the molecular weight was highly significant.

Polyacrylamide gel electrophoresis

In nondissociating discontinuous PAGE system the procedure of Davis (1964) with 5% (w/v) acrylamide (5% T; 2.6% C)* in the running gel at pH 8.9 (Tris-glycine buffer), and 2.5% acrylamide in the spacer gel was used. 100 µl aliquots of crude extract or eluates from a column of Sephadex G-200, containing 250-350 µg of protein, were applied to the spacer gel. Cathode buffer was twice as much concentrated as anode one. Electrophoresis was carried out for 3.5 h at 4 mA per tube, after initial 15 min period at 2 mA per tube. Protein bands were visualized by positive staining with 0.04% Coomassie Brillant Blue G-250 in 3.5% perchloric acid (Reisner et al., 1975).

The running gel in the dissociating system contained 10% acrylamide with 2,7% N,N′-methylebisacrylamide cross linker, and 1.0% SDS (sodium laurylsulphate) in 0.1 M phosphate buffer, pH 7.0 (Eastman SDS). Protein samples were boiled for 5 min in 0.01 M phosphate buffer containing 1% SDS, 1% 2-mercaptoethanol and 8 M urea, and dialyzed for 12 h against 0.01 M phosphate buffer supplemented with 0.1% SDS and 0.1% 2-mercaptoethanol. 20 µl aliquots containing 18-32 µg of protein were applied to the gel. 0.1 M phosphate buffer containing 1% SDS at pH 7.0 was used as electrode buffer. Electrophoresis was carried out for 12 h at 2 mA per tube. Protein bands were stained with 0.25% Coomassie Brillant Blue G-250 in a mixture of 7% acetic acid in 50% methanol.

Molecular weights were read from a curve of relative electrophoretic mobilities (Rm) of protein standards: bovine serum albumin, MW 68 000; ovoalbumin, MW 45 000; chymotrypsinogen, MW 27 500; RNase A, MW 13 700; and cytochrome C, MW 11 700. All protein standards were obtained from Serva Feinbiochemica (Heidelberg) and reagents for PAGE and PAGE SDS were obtained from Eastman Kodak and Co., Rochester, N. Y.

Gels were scanned at 600 nm in Carl Zeiss (Jena) densitometer Eri 65m with a home made attachement for polyacrylamide slabs.

* $T = \frac{a+b}{m} \cdot 100\%$; $C = \frac{b}{a+b} \cdot 100\%$, where a = acrylamide (g); b = bisacrylamide (g); m = volume of buffer (ml).

RESULTS

Four protein fractions were eluted from a column of Sephadex G-200. Fraction A, eluting in V_o (MW 540 000 daltons), was heavily contaminated with non protein compounds as inferred from a low A 280/A 260 nm ratio (Table 1). Majority of protein was eluted in a fraction B (MW around 350 000), followed by minor fraction C and D (MW's around 100 000 and 22 000, respectively). There were also fractions *e* and *f* which overlapped and contained low molecular non protein material. Fractions A and B overlapped.

Table 1

UV absorbance characteristics and apparent molecular weight of salt extractable soybean seed protein fraction separated on a Sephadex G-200 column. In parentheses results of similar analyses carried out by Sabir, Sosulski and MacKenzie (1973) are given. Fractions A and B in this study overlapped

Fraction	λ max, nm	$\frac{A\ 280}{A\ 260}$	Apparent MW, daltons
A	260 (260)	0.70 (0.90)	>540 000 (600 000)
B	278 (280)	1.54 (1.30)	350 000 (400 000)
C	276 (275)	1.70 (1.00)	100 000 (120 000)
D	270 (275)	1.00 (1.10)	22 000 (30 000)

Aliquots of fractions A-D were subjected to PAGE under nondissociating conditions. The gels were intentionally overloaded to reveal minor protein fractions. 18 protein bands were detected in a crude extract, the major fractions being bands No. 3, 4, 7 and 8 (Fig. 1). These bands possibly correspond to bands No. 1-4 in a similar study performed by Hill and Breidenbach (1974) who detected only 5 fractions. The

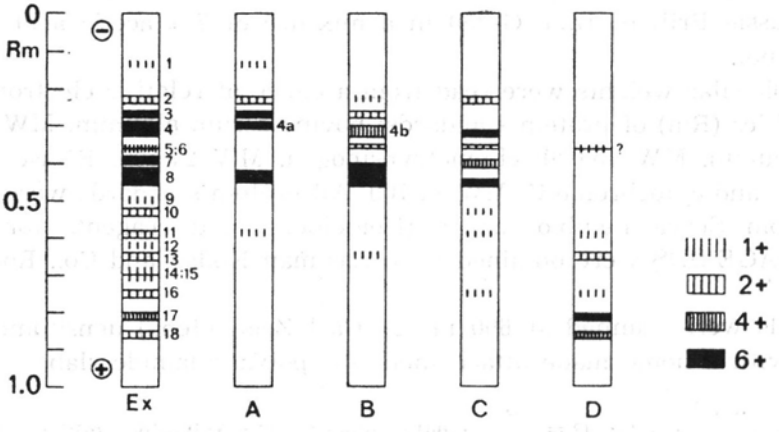

Fig. 1. Electrophoretic patterns of salt extractable proteins under nondissociating conditions. Ex — total extract; A, B, C, and D — Sephadex G-200 fractions

bands No. 4 and 8 were present in fractions A, B and C. The band No. 4 seems to be heterogenous as it tends to separate to bands No. 4a and 4b in the fractions A and B, respectively.

Band No. 7 was not detected in the fraction A. Bands 8 and 17 were the major ones in fractions C and O, respectively (Fig. 1).

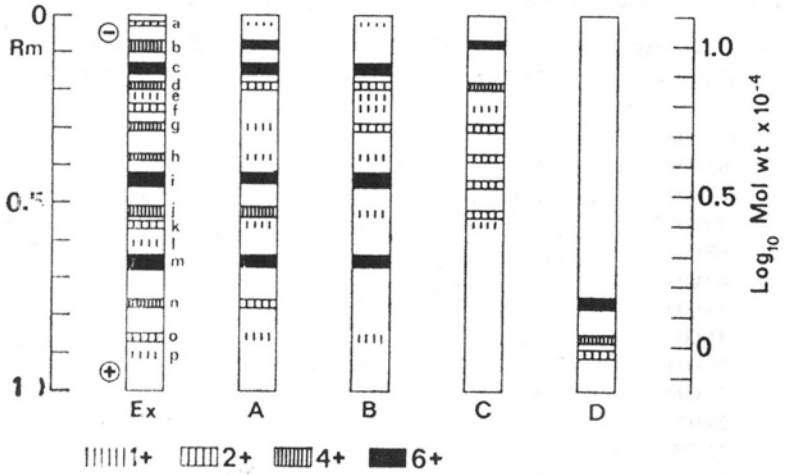

Fig. 2. Electrophoretic patterns of salt extractable proteins under dissociating conditions (SDS PAGE). Abbreviations as in Fig. 1

Sixteen bands *a-p* were detected when total protein extract was subjected to PAGE SDS (Fig. 2). The main bands were the bands *c*, *i* and *m*, followed by *b*, *d* and *j* (Fig. 3). The band *b* (MW 95 000) was detected in fractions A and C, but not in a fractions B. In the latter fraction the bands *c*, *i* and *m* were the major ones. Molecular weights of these proteins were determined for around 80 000, 36 000 and 18 500

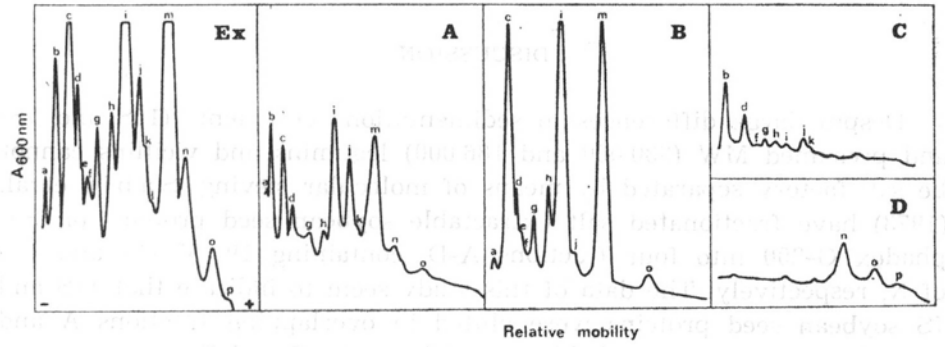

Fig. 3. Relative electrophoretic mobilities of salt extractable soybean seed proteins under dissociating conditions. Scannings were made at 6000 nm. Abbreviations as in Fig. 1

daltons, respectively (Table 2). It seems that the bands *c*, *i* and *m* correspond to bands No. 6 (MW 75 000), 17 (MW 36 500), and 22 (MW 18 500) on electropherograms presented by Savoy (1977).

Table 2

Molecular weights of protein bands detected in SDS PAGE. Relative intensity of the bands in chromatographic fractions A—D (Table 1) is denoted with 1—6+ (*cf.* Fig. 3).

Band	Apparent MW, daltons	Presence in Sephadex G-200 fractions			
		A	B	C	D
a	122 000	±	±	–	–
b	95 000	6+	–	6+	–
c	80 000	4+	6+	–	–
d	70 000	+	2+	3+	–
e	67 000	–	±	–	–
f	60 000	–	±	±	–
g	53 000	±	2+	+	–
h	41 000	+	2+	+	–
i	36 000	6+	6+	+	–
j	27 000	4+	±	+	–
k	24 000	±	–	±	–
l	21 500	–	–	–	±
m	18 500	6+	6+	–	–
n	13 700	+	–	–	6+
o	11 000	+	+	–	3+
p	9 500	–	–	–	+

Protein with MW 27 000 (*j*) was characteristic for the fraction A. It was virtually absent in other fractions. Bands *b* and *d* (MW 95 000 and 70 000, respectively) were the main ones in a fraction C in which bands *c* (MW 80 000) and *m* (MW 18 500) were absent. Band *n* (MW 13 700) was dominant in the fraction D; it was present also in a fraction A, and absent in fractions B and C.

DISCUSSION

Despite large differences in sedimentation coefficients (11S and 7S) and presumed MW (330 000 and 186 000) legumins and vicillins cannot be satisfactory separated by means of molecular sieving. Sabir et al. (1973) have fractionated salt extractable soybean seed proteins on Sephadex G-200 into four fractions A-D, containing 19, 57, 11 and 1% of N, respectively. The data of this study seem to indicate that 11S and 7S soybean seed proteins were eluted in overlapping fractions A and B. 2.8S proteins were probably eluted in peaks C and D.

Results of electrophoretic analyses in nondissociating system support a general view that a limited number of proteins make up the bulk of

the protein in soybean seed (Hill and Breidenbach, 1974). Nevertheless, many other proteins are present in minor amounts; they possibly play catalytic functions (*cf.* Grzesiuk and Łuczyńska, 1972; Kulka and Grzesiuk, 1978; Millerd, 1975; Sabir et al., 1973).

Available data are inconsistent as concerns the MW of the subunits of 7S globulin fraction of soybean seed. In ultracentrifuge studies the MW of the subunits was determined for 23 000 and 35 000 (*cf.* Darbyshire, et al. 1976; Kulka and Grzesiuk, 1978). SDS PAGE gave much higher estimates of 23 000, 51 000 and 81 000 (Masaki and Soejima, 1972). This study revelated that three major protein bands have MW around 80 000, 36 000 and 18 500 daltons. The protein with the molecular weight of around 80 000 can be, thus, regarded as one of the major component of the storage proteins of soybean seed. Basing on the data presented by Masaki and Soejima (1972) it can be suggested that protein with MW 80 000 (band *c*) is a product of dissociation of the 7S globulin.

It has been suggested that glycinins (11S) molecule is built up of equimolar amounts of acidic (MW 37 000) and basic (MW 22 000) subunits (Catsimpoolas *et al.*, 1967; Catsimpoolas, 1969). This implies that in SDS PAGE there should be bands corresponding to these values of MW. Proteins *i* (MW 36 000) and *m* (MW 18 500) were the most intense in fractions A and B. These bands are possibly components of the 11S storage globulins.

Recently Savoy (1977) detected 28 protein bands on electropherograms of each of 21 different soybean seed varieties. Sixteen bands detected in this study fit well with Savoy's data. Protein spectrum in the seed of soybean cultivar. 'Warszawska' seems to be similar to protein spectra of other soybean varieties.

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Elektroforeza białek nasion soi w żelu poliakrylamidowym

Streszczenie

Białka nasion soi odm. 'Warszawska' ekstrahowano 2.5% zbuforowanym roztworem NaCl, chromatografowano na żelu Sephadex G-200 i analizowano elektroforetycznie. Wykryto 18 pasm białkowych na elektroferogramach w układzie niedysocjującym oraz 16 pasm w układzie dysocjującym (SDS PAGE). Ciężar molowy głównych pasm białkowych wynosi 18 500, 36 000 i 80 000 daltonów.