

Studies on Soybean Seed Proteins by Means of Disc Electrophoresis

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Abstract. Three protein fractions (albumins, globulins and glutelins) of cotyledons of ripe and dormant soybean seeds were analysed by a modified polyacrylamide disc electrophoresis method. All fractions were shown to be multicomponent and easily characterized by their "protein electrophoresis spectra". A method of characterizing proteins by their relative electrophoretic mobility (R.E.M.) by comparison with a new nontoxic dye as a tracer is employed for a preliminary identification of proteins. Specificity of protein complement was investigated in view of the authors, further studies on dynamics of specific proteins in the course of ripening and germination of soybean seeds.

Being rich in soluble reserve seed protein, plants of the *Papilionaceae* family are often suitable sources of proteins for plant protein studies. However, in spite of protracted studies on proteins of legumes these substances are still insufficiently known (BLAGOVESHCHENSKY 1967). Under native conditions seed proteins exist as quite complex mixtures or compositions of multiple proteinaceous components. Sets of such components and their relative concentrations are more or less different at various phases of seed development (GHETIE and BUZILA 1964, KLOZ, KLOZOVÁ and TURKOVÁ 1966, SAFONOVA 1967).

In order to investigate contents and properties of multicomponental protein complexes in seeds in connection with physiological problems it is expedient to use new methods of biochemical analyses. Some of them have high discriminative potential and offer possibilities of simultaneously following several protein components, even those of very similar properties. It is very desirable to quickly identify multiple protein components by their characteristic features and small amounts of these purified proteins could be obtained for further study. Methods of modern protein chemistry based on differential migration of macromolecules are especially capable of meeting these requirements. The most convenient and economical analytical methods in this group are polyacrylamide gel electrophoretic methods, especially disc electrophoresis (DAVIS 1964).

BRIGGS and MANN (1950) were the first to publish electrophoretic studies on soybean seed proteins. They found seven protein components in a water extract from the seeds and several proteins were observed in globulin (glycinin) preparations made by a series of methods the number of constituents depended upon the particular preparative procedure. SMITH, SCHUBERT and BELTER (1955) separated soybean proteins into 2 fractions: a soluble one at pH 4.5 and an insoluble fraction under the same conditions. Five protein components were found in each fraction. Two proteins exhibited the same mobility so that the total number of components was 8. However SZYLAGYI (1965) after separation of soybean proteins into albumins and globulins at pH 4.5 found 9 protein components in the albumin fraction. Thus a high multiplicity of protein components in soybean seeds was becoming more and more evident.

Perhaps, these features of soya proteins could be demonstrated still more clearly, if polyacrylamide gel electrophoresis were used because of its superior resolving power. For example FOX, THURMAN and BOUTLER (1964) were able to find up to 23 separate proteins in the albumin fraction of pea seeds.

Therefore we thought a first step in our studies on dynamics of proteins in ripening and germinating soybean seeds should be an application of a disc electrophoresis method to reveal a real multiplicity of protein complexes in dormant ripe seeds. Further observations on dynamic protein patterns in soybean seeds were expected to be useful for an appreciation of the importance and probable function of a least several main protein components in these seeds.

Material and Methods

Separated cotyledons of ripe dormant seeds of soybean variety Primorskaya 529 (recolcted in 1965) were prepared by hand and milled in an electric coffee mill. The resulting meal was defatted with light petroleum at room temperature by means of several washings and decantations of the extractant layer. Defatted meal was dried on filter paper sheets and kept cool.

Proteins were extracted twice with a 15-fold volume of phosphate buffer (pH 7.0) with 10% NaCl added. Extractions were performed for 3 hours at 4° and with periodical hand stirring. The extracts were clarified by centrifugation at 5000 rev./min in a water-cooled centrifuge. Proteins were then totally sedimented by saturating the extract with pulverized solid $(\text{NH}_4)_2\text{SO}_4$. The resulting sediment was separated by centrifugation, then suspended in a minimum of water and dialysed in Visking cellophane tubing against distilled water for 3 days. Albumins were then separated from globulin by acidifying the dialysed solution with diuted acetic acid up to pH 4.5 (BOETGER 1958).

Glutelins were extracted from the insoluble residue of seed meal with 0.2% solution of NaOH at 4°. The extracts were then dialysed. Each protein fraction was lyophilised and analyzed by a disc electrophoretic method described separately (SAFONOV and SAFONOVA 1969, in press).

Electrophoretic separations were performed in 7.5% polyacrylamide gels with basic and acidic buffer systems, as described by DAVIS (1964) and REISFELD, LEWIS and WILLIAMS (1962), but with several modifications

important for plant protein separations. The sample gel was replaced with 6% linear polyacrylamide solution containing the corresponding electrode compartment buffer. Protein zones were stained with 0.1% solution of xylenebrillantcyanin G (Sandoz, Basel) and were observed as very bright and clear bands even at the lowest component concentrations (less than 10 μ g per zone). Excess dye was easily washed out with a mixture of methanol, water and concentrated acetic acid (10 : 30 : 1). A food dye — Sunset yellow FCF Supra (Pointing, Hexham, England) was used as a tracer during electrophoretic separations for it obviously did not interact with acidic proteins and migrated as a single compact zone in front of all the proteins, which then could be characterized by their relative electrophoretic mobility (R.E.M.).

A simple electrophoretic apparatus made in The Plant Physiology Institute was used throughout because it had a cooling system with melting ice and an efficient buffer circulation system (SAFONOV and SAFONOVA in press).

Results and Discussion

It appeared, after electrophoresis in the alkaline gel, that soybean cotyledons possessed numerous (up to 17) albuminous components migrating to the anode (Table 1). In the acidic gel 5 protein bands moving to the cathode were found. It was of interest to determine whether all these 21 proteins were different kinds of molecules or if there were any proteins among them which exhibited basic properties in the acidic buffer and acidic ones in the alkaline buffer. For this purpose a brief additional experiment was performed as follows.

The whole set of protein zones which migrated to the anode in the alkaline buffer system were removed from the gel (not fixed and not stained, of course) with the tris — glycinate buffer used normally for electrode vessels. This extract was introduced into electrophoretic tubes filled with pure acidic gel for subsequent electrophoretic separation. Two protein zones were observed in the electrophoregrams obtained. These zones were almost identical on the basis of their R.E.M. (0.32 and 0.52) with 2 normally seen acidic components (R.E.M. 0.33 and 0.58). So it would be reasonable to admit that the albumin fraction of soybean cotyledons consists preferentially of acidic proteins (not less than of 14 components), but also includes 3 basic protein components and 2 approximately neutral proteins whose mobility changes readily in accordance with pH of solutions.

Globulins were separated into 11 anodic components when subjected to electrophoresis in the alkaline buffer gel (Table 1) in the acidic gel there were 7 cathodic components. In the alkaline gels containing 2 M urea globulin components separated less clearly, globulin patterns were clear after electrophoresis in acidic gels with 2 M urea. Obviously, urea induced a dissociation of some globulins into their subunits because 4 additional protein bands were seen under the respective electrophoregrams (Table 2). A dissociation of leguminous globulins under the influence of urea has been observed by several other authors (FOX, THURMAN and BOULTER 1964, WEINTRAUB and SHUTOV

1966). When globulins were subjected to electrophoresis in the acid gel without urea a wide protein zone (very near to the start) was observed which almost disappeared in the presence of urea. It would be possible to admit that there existed a protein (or proteins) dissociable into subunits with urea (Table 1). Several globulin bands disappeared as well from the gel electrophoregrams (Table 1) when urea was present in the alkaline gel (for example, components with R.E.M. of 0.05; 0.39; 0.44; 0.64). At the same time there

Table 1

Relative electrophoretic mobility (R.E.M.) of anodic protein components of soybean cotyledons in alkaline buffer gel.

Albumins	Globulins	Globulins in the presence of 2M urea	Glutelins
0.02	—	—	—
—	0.05	—	0.06
0.15	0.14	0.13	0.11 0.13
0.18	0.20	0.18	0.17
—	0.25	0.23	—
0.28	—	—	0.28
0.35	0.33	0.33	0.33
0.38	0.39	—	0.39
0.42	0.44	—	—
0.47	—	—	—
0.52	—	0.50	0.50
0.54	—	0.55	—
0.57	0.56	—	—
0.68	0.64	—	0.63
0.78	—	0.73	0.73
0.81	—	—	—
0.89	0.86	0.88	—
0.95	—	—	—
1.00	1.00	1.00	1.00

(Sunset yellow FCF Supra is used as the tracer with R.E.M. = 1.00)

appeared additional protein zones with R.E.M. of 0.50; 0.73; 0.88. We believe that in this case a similar dissociation of protein components into subunits with higher relative mobility and, perhaps smaller molecular dimensions occurs. A certain role of hydrogen bonds in such association — dissociation processes could be expected.

Glutelins of soybean cotyledons were presented by 11 anodic components when separated in the alkaline gel (Table 1). In the acidic gel there were 7 cathodic components (Table 2).

It can be seen from the results obtained that the polyacrylamide gel electrophoresis used made possible sufficiently more detailed separations of protein fractions of soybean cotyledons into characteristic components. The protein fractions analysed (albumins, globulins and glutelins) are undoubtedly complex mixture of multiple and various protein components. Every fraction contains its inherent proteins with a characteristic electrophoretic mobility

(R.E.M.). If the relative concentration (evaluated visually or otherwise on the basis of staining intensity) is also considered a specific and very characteristic "protein electrophoretic spectrum" is observable for each of the fractions studied. If some additional factors (for example urea or gel concentration) are involved a still more specific identification and study of a particular fraction can be performed.

There are several components in common for all or some of the protein fractions studied. Perhaps, they are almost inevitable products of fractions obtained by a solubility fractionation. It may be supposed that any kind of salt — like or adsorptive weak interaction plays its role in such cases,

Table 2

Relative electrophoretic mobility (R.E.M.) of cathodic protein components of soybean cotyledons in acidic buffer gel.

Albumins	Globulins	Globulins in the presence of 2M urea	Glutelins
—	—	0.14	—
—	0.17	—	—
0.21	0.20	0.20	0.23
—	0.25	0.26	0.26
—	0.30	—	0.29
0.33	—	0.34	—
—	0.36	0.35	—
—	—	0.40	0.39
—	0.45	0.43	0.42
—	—	0.49	—
0.58	—	0.57	0.55
—	0.62	0.60	—
0.67	—	0.66	—
—	—	—	0.70
0.79	—	—	—

(Methylene blue is used as the tracer with R.E.M. = 1.00).

especially in salt — free solutions. It appears from our experience that more pure preparations of albumins and globulins are obtainable when a total extraction of proteins with a high concentration (10%) salt solutions is first applied followed by a dialysis fractionation. On the contrary a traditional stepwise solubilization of albumins and globulins often results in more heavily contaminated preparations of seed proteins.

A simultaneous polyacrylamide gel electrophoresis in acidic and alkaline buffer systems and the method of a protein zone identification used seem to be useful tools for a short — time preliminary evaluation of a real complexity of protein fractions, their comparisons and evaluations of purity of preparations obtained. Further studies on soybean seeds protein fractions from dynamic aspects perhaps, will enable us to interpret the results obtained more comprehensively.

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V. I. SAFONOV*, M. P. SAFONOVA*, N. A. NARBUT**, *Ústav fyziologie rostlin K. A. Timirjazeva AV SSSR, Moskva, **Ústav biologie a půdoznalství Sibiřského oddělení AV SSSR, Vladivostok: **Studium bílkovin semen soje metodou diskové elektroforesy v polyakrylamidovém gelu.** — Biol. Plant. 11 : 183—189, 1969.

Byly studovány tři bílkovinné frakce (albumíny, globulíny a glutelíny) děloh zralých klidových semen soje modifikovanou metodou diskové elektroforesy. Bylo zjištěno, že všechny frakce jsou mnohokomponentní a dobře charakterizované svým „bílkovinným elektroforetickým spektrem“. Pro předběžnou identifikaci bílkovin byla použita metoda jejich charakterisování podle jejich relativní elektroforetické pohyblivosti při porovnání s novým netoxickým potravinovým barvivem „Sunset Yellow FCF Supra“, jejíž pohyblivost se pokládá za 1. Specifičnost bílkovinného komplexu semen soje byla studována ve vztahu k dalším pracem autorů týkajících se dynamiky specifických bílkovin při dozrávání a klíčení semen soje.

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Модифицированным методом диск—электрофореза в полиакриламидном геле были изучены три белковые фракции (альбумины, глобулины и глютелины) семян зрелых покоящихся семян сои. Как было показано, все фракции являются многокомпонентными и довольно характерными, судя по их «белковым электрофоретическим спектрам». Для предварительной идентификации белков использован метод характеристики белков по их относительной электрофоретической подвижности (О. Э. П.) при сравнении с новой нетоксичной пищевой краской «Sunset yellow FCF supra», взятой в качестве метчика с подвижностью, принимаемой за 1. Специфичность белкового комплекса семян сои изучалась в связи с дальнейшими работами авторов по динамике специфических белков в процессе созревания и прорастания семян сои.