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A New Electrophoresis Method and its Application for Revealing the Heterogeneity of the Chromatographically Homogeneous Fractions of Soybean Proteins

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Summary

A simple horizontal slab disc-electrophoresis using discontinuous buffers of Davis and Ornstein was designed for direct analysis of the samples eluted from gel column. This method unexpectedly revealed a distinct order of elution of the five electrophoretic components of the 7S soybean globulin from Sepharose gel column. Therefore, a combination of gel chromatography and disc-electrophoresis would give sufficient evidence for the existence of multiple components which were not resolved by gel chromatography no ascertained by electrophoresis alone.

Furthermore, slab electrophoresis using dissociating buffer (5 M urea, 30% acetic acid) allowed the investigation of the subunit composition of various fractions obtained by column chromatography. Even though it was not possible to state the subunit composition of the individual components, this technique demonstrated that the five 7S components detected by gel chromatography and disc-electrophoresis were made up of two major and two minor types of subunits in different ratios.

In the column chromatography of proteins, several components of the sample are often not clearly resolved and they appear as a single peak on a chromatogram. For detecting these different components, the disc-electrophoresis method of Davis (1) and Ornstein (2) proved to be very effective. But the dissociation-association of proteins which accounts for the multiple bands by disc-electrophoresis may not be ruled out. Furthermore, in the case of gel cylinders, protein bands of nearly similar mobility may be incomparable.

In this paper, we describe a simple slab-electrophoresis to which the effluents from a column can be applied directly. By this method, the distribution of the electrophoretically heterogeneous components across the elution peak can be detected and evidence for the existence of multiple components can be revealed.

This method was used to investigate the distribution of the 7S and 11S soybean globulins by gel chromatography on Sepharose 6B. The two major reserve proteins

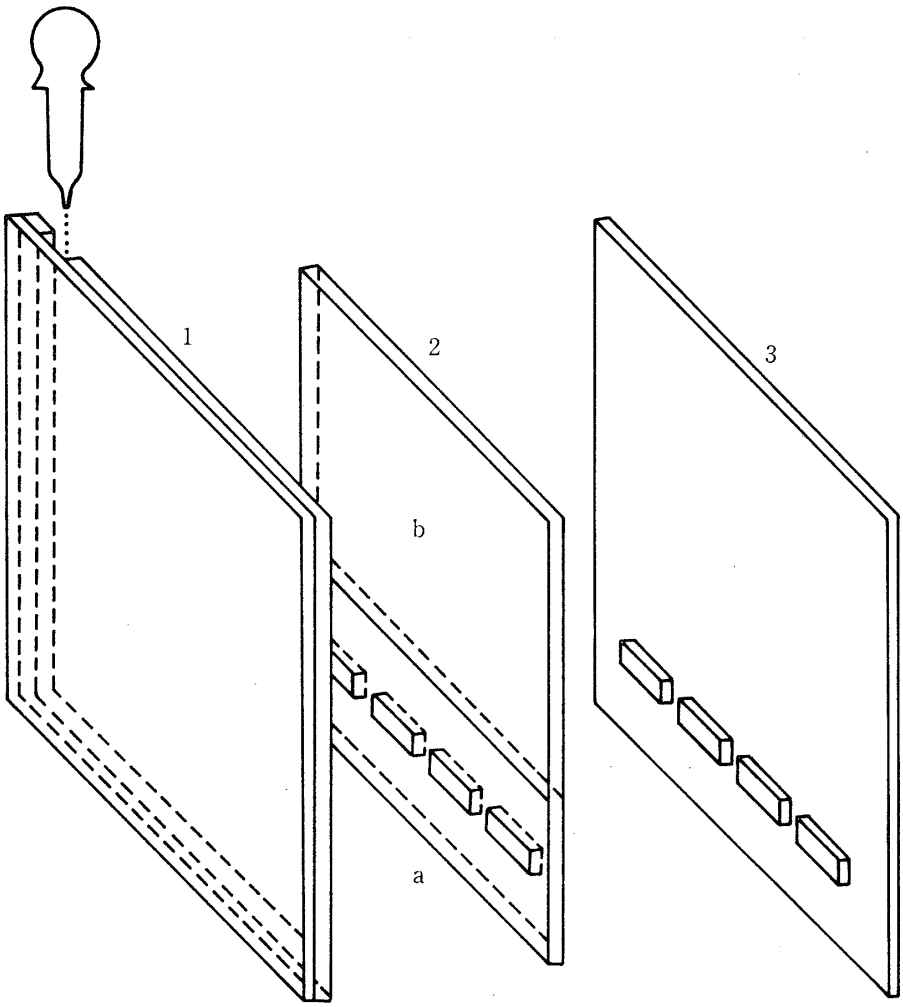

Fig. 1. Design of the discontinuous acrylamide-gel slab. 1: acrylic plastic cell (14×21 cm), 2: prepared discontinuous acrylamide gel with spacer gel (a) and separating gel (b), 3: acrylic plastic cover with molds for sample slots (0.14×0.25×1.0 cm).

which comprise 70% of the total soybean proteins, were reported to be eluted together from Sephadex G-200 gel column (3, 4) even though they are different in molecular weight and sedimentation constant. The simple electrophoresis method proved that the 7S globulin eluted with the 11S from gel column was composed of five components which had different electrophoretic mobility and which were distributed on a gel chromatogram in a distinct order.

Methods

A 14×21 cm acrylic plastic cell is made from a 3 mm-thick acrylic sheet and 1 cm-wide acrylic strips. Molds for sample slots (0.14×0.25×1 cm) are cut from a 1.4 mm-thick acrylic plastic plate and set on a cover plate with adhesive at intervals of 5 mm (Fig. 1). Rectangular tanks are used as electrode reservoirs.

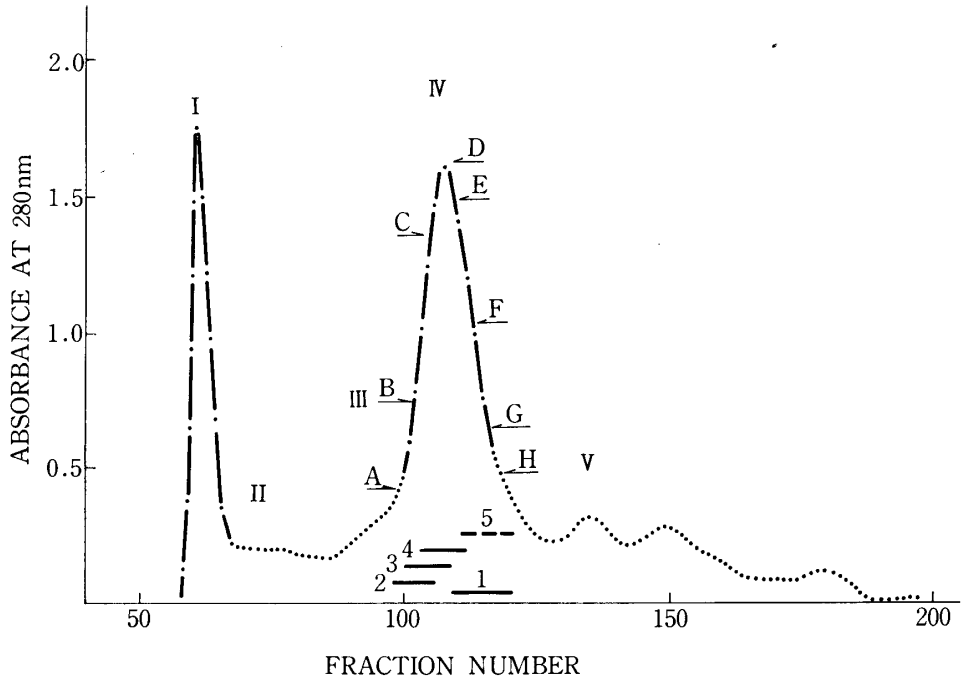

Fig. 2. Elution profile for 400 mg of soybean globulins with 4.80 ml fractions from Sepharose 6B column (2.5×183 cm). Column was eluted with 0.5 ionic strength standard phosphate buffer. Flow rate was 14 ml/hr. Fraction A to H were subjected to the horizontal slab-disc-electrophoresis. The bars under the peak IV represent the elution patterns of the 7S components detected in Fig. 3.

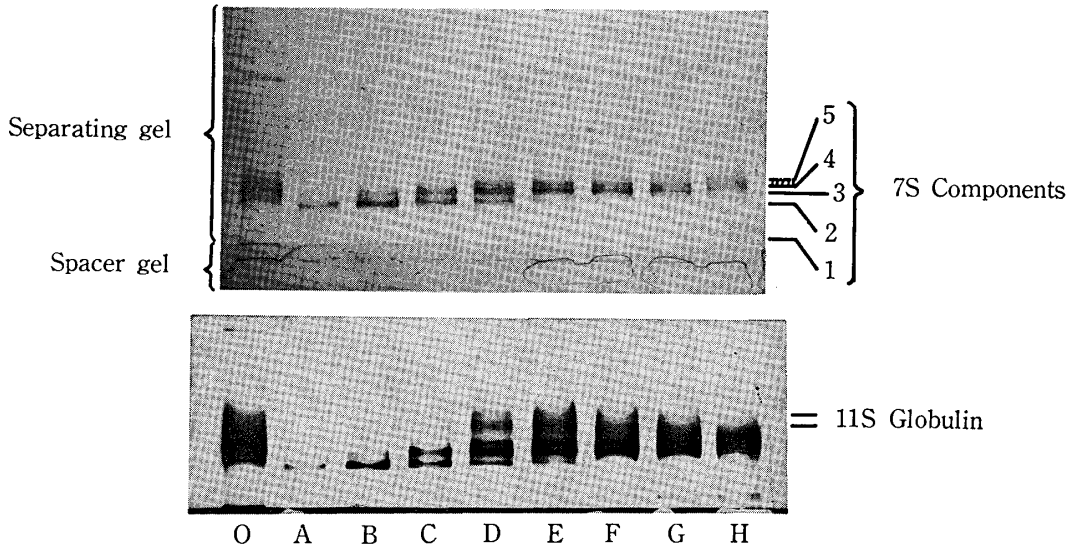

Fig. 3. Slab-disc-electrophoresis of the selected fractions in Fig. 2. Spacer gel: 4% acrylamide, 10% bis/acrylamide, 0.06 M Tris-HCl (pH 6.7). Separating gel: 6% acrylamide, 3% bis/acrylamide, 0.38 M Tris-HCl, 0.02 M ß-mercaptoethanol (pH 8.9). Electrode buffer: 0.025 M Tris-Glycine (pH 8.3). Gel stained with periodic acid-Schiff (upper) and subsequently stained with Amido Black (lower).
O: soybean globulins. A to H: selected fractions in Fig. 2.

Preparation of the Slab-Discontinuous-Gel

For preparation of gel, the acrylic plastic cell is rimmed with vaseline and the cover is firmly fixed with steel clips leaving a clearance of 0.5 mm between the bottom of the slots and that of the cuvette. The spacer gel solution (1) containing 4% acrylamide and 10% bis/acrylamide is poured into the block through a hole at a coner (Fig. 1) to a level 7 mm above the molds for sample slots. The block is fixed in a vertical position. Water layering is facilitated with the use of a water reservoir at an appropriate hydrostatic pressure (about 10 cm). Following the photopolymerization of the spacer gel, the supernatant water is pipetted off and the remaining spacer of the block is filled with the separating gel solution (1) containing 6% acrylamide and 3% bis/acrylamide. The gel block is uncovered and the slits are filled up with the samples (35 to 40 μl) eluted from column. The surface of the gel is covered with a thin polyvinyl sheet (saran wrap), avoiding air bubbles. All the above procedure can be performed within 2 hr and the gel slab (0.3×12×19 cm) allows simultaneous analysis up to 11 samples.

Preparation of the Slab-Continuous-Gel

The acrylic plastic block is filled with the separating gel solution (8% acrylamide, 3% bis/acrylamide, 5 M urea, 30% acetic acid, 0.4% ammonium persulfate and 0.5% TEMED, pH 3.0). Polymerization is accomplished as the gel block is incubated at 40° for 40 min. Excess catalysts are removed by pre-electrophoresis (30 mA, 10hr) with methylene blue as a dye-marker. This method was applied to investigate the subunit composition of the 7S components eluted from gel column.

Electrophoresis

An electrical contact to the gel plate is formed using 5 to 6 layers of filter paper. Two platinium electrodes (21 cm in length) are placed parallel to the edges of the gel plate. A current of 10 mA is applied for the first 20 min, then a constant current of 35 mA is maintained. Running time is usually 3 to 4 hr for the slab-discontinuous gel, and 12 hr for the slab-continuous gel. Otherwise, electrophoresis in the dissociating buffer is carried out at a constant current of 50 mA for 6 hr.

Results and Discussion

Fig. 2 shows the elution profile of 400 mg of soybean globulins from Sepharose 6B column (2.5×183 cm) eluted with 0.5 ionic strength standard phosphate buffer (5). Of the 4 components of soybean proteins (15S, 11S, 7S, and 2S), two major components (11S and 7S) were eluted in the main peak (peak IV) (6). Peak I had a relatively high optical density that was due mainly to the visible turbidity because little protein was recovered from it. The 15S and 2S components were eluted in

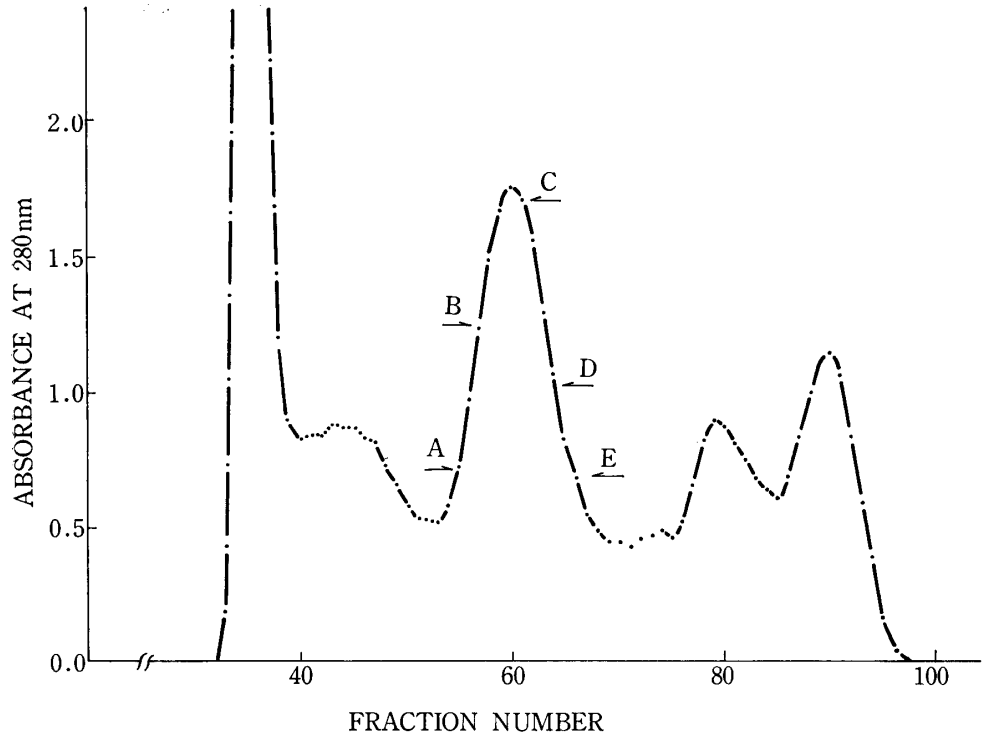

Fig. 4. Elution profile for 1500 mg of the crude 7S fraction with 20 ml fractions from Sepharose 6B column (4×140 cm). Column was eluted with 0.5 ionic strength standard phosphate buffer. Flow rate was 50 ml/hr. Fractions A to E were subjected to the electrophoresis in AU solvent.

peak III and peak V, respectively (unpublished data). The selected fractions (Fig. 2, fraction A to H) were subjected directly to the slab-disc-electrophoresis. The gel plate was stained with the combined periodic acid-Schiff (7) and Amido Black. Fig. 3 shows clearly a different distribution of the electrophoretic components of the 7S and the 11S globulin (dimer and monomer) by gel chromatography even though the elution profile of the peak IV seemed to be homogeneous. The bars under the main peak (Fig. 2) represented the elution patterns of the 7S components. It was noted that the distribution of the 11S globulin across peak IV was uniform and that of the 7S components was not: three 7S components (components 2 to 4) were found to be eluted with or slightly in front of the 11S; the two other components (1 and 5) were predominant toward the trailing edge of the peak. All these five components developed a positive glycoprotein staining reaction. This indicated that these components are not the polymerized forms of the 11S globulin which showed a negative reaction for this staining method (unpublished data). Because of the distinct elution order of the 7S components, all five 7S components may represent the multiple 7S globulins in soybean proteins.

More evidences for the heterogeneity can be obtained as one investigates the subunit composition of the sample eluted from column. For this purpose, the crude 7S fraction (8) was applied to Sepharose 6B column. Selected fractions

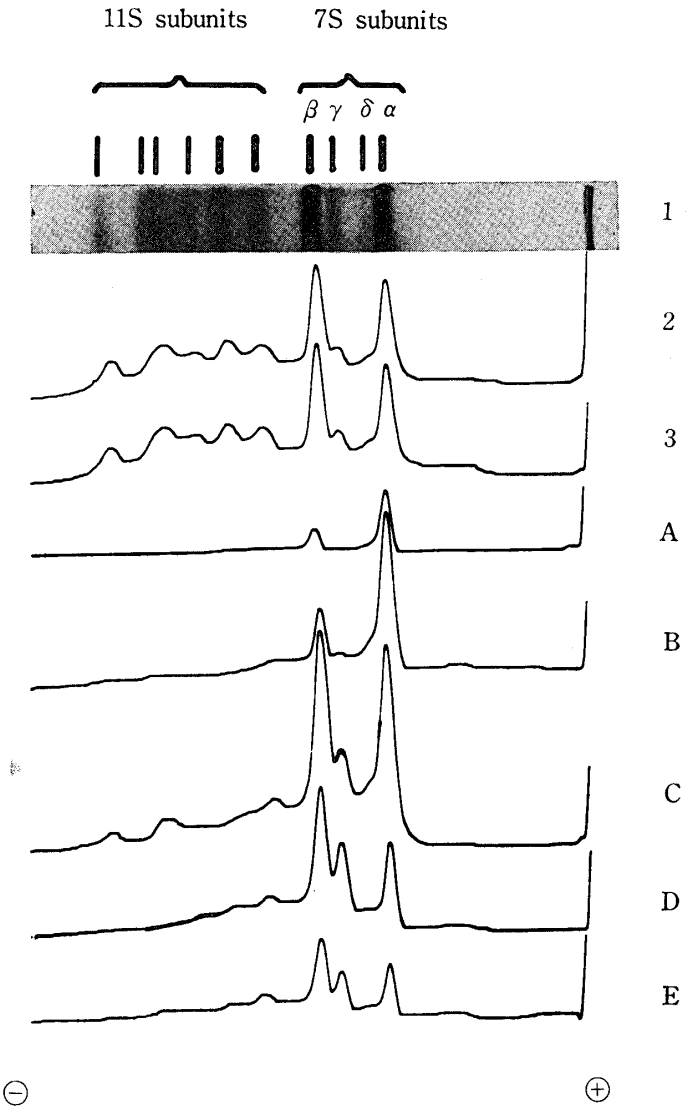

Fig. 5. Electrophoresis patterns and densitometer tracings of soybean globulins (1 to 3) and the selected fractions in Fig. 4. (A to E). All the samples were treated in 8 M urea, 0.2 M ME for 1 hr; then acetic acid was added to a concentration of 30% (v/v). The gel plate contained 8% acrylamide, 3% bis/acrylamide, 5 M urea and 30% acetic acid (pH 3.0). The electrode buffer was 10% acetic acid. Electrophoresis was carried out at a constant current of 50 mA for 6 hr. Gel stained with Coomassie Blue.
1 and 2 were soybean globulins in 0.5 μ phosphate buffer. 3 was soybean globulins without the phosphate buffer.

(Fig. 4, fraction A to E) were reduced in 8 M urea, 0.2 M β-mercaptoethanol (ME) for 1 hr (calculated urea and ME were added to 0.5 ml eluent). Acetic acid was added to the sample solution to 30% (v/v) then, treated samples were subjected to the slab-gel electrophoresis in urea-acetic acid system. Fig. 5 shows the electrophoresis patterns of soybean globulins (upper rows). The 7S globulin fraction appears to be composed of two major (α and β) and two minor types of

subunits (γ and δ). All the four subunit bands gave a positive staining for glycoprotein. Densitometer tracings of the electrophoresis patterns indicate the difference in subunit composition of the different 7S fractions (Fig. 5, A to E): the leading half of the peak (Fig. 4, A and B) which contained mainly the 7S components 2 and 3 was rich in α-type subunits (Fig. 5, A and B); the trailing half of the peak in which 7S components 1 and 5 were predominant, had more ß-type than α-type along with γ and δ-type subunits (Fig. 5, C and D). Because the five 7S components were uncompletly resolved by gel chromatography, only the differences in the ratio of the major subunits were observed. Nevertheless, this result indicated that all five 7S components were made up of two major and two minor types of subunits. Although Catsimpoolas et al. (9) reported that the 7S protein (named γ-conglycinin) was dissociated into 9 subunits detected by electrophoresis in PAMU solvent (phenol-acetic acid-mercaptoethanol-urea), Koshiyama (10) in an identical experiment, detected only two major and three minor bands. Electrophoresis in 5 M urea-30% acetic acid (AU solvent) gave sharper bands than those obtained with PAMU solvent, with similar resolution (11). Therefore, AU solvent was suitable for this investigation. Because excess catalysts were already removed by pre-electrophoresis, incorporation of mercaptoethanol into polyacrylamide gel was unnecessary, provided the samples were treated previously with reducing agents. Electrophoresis in AU solvent may be preferable to fractionate the subunits which are not well resolved by alkali-system electrophoresis (like subunits of the 7S soybean globulin). The size of the subunits seemed to be the predominant factor for their electrophoretic mobility in AU-system electrophoresis. It was found that the α and β-type subunits had different molecular weights (about 57,000 and 43,000, respectively-unpublished data). This was consistent with the previous report which indicated that the molecular weight of polypeptides could be estimated with any degree of accuracy by electrophoresis in acetic acid-urea solvent (12).

In conclusion, the horizontal slab-disc electrophoresis described here allows the investigation of the distribution of multiple electrophoretic components across a single peak eluted from gel column. This investigation may give sufficient evidence for the heterogeneity of the sample which is not ascertained alone by electrophoresis. Furthermore, electrophoresis in AU solvent may reveal the heterogeneity at subunit level.

References

1) Davis, B.J., *Ann. N.Y. Acad. Sci.*, **121**, 404 (1964)
2) Ornstein, L., *Ann. N.Y. Acad. Sci.*, **121**, 321 (1964)
3) Hasegawa, K., Kusano, T., and Mitsuda, H., *Agr. Biol. Chem.*, **27**, 878 (1963)
4) Koshiyama, I., *Agr. Biol. Chem.*, **33**, 281 (1969)
5) Wolf, W.J., and Briggs, D.R., *Arch. Biochem. Biophys.*, **63**, 40 (1956)
6) Thanh, V.H., Okubo, K., and Shibasaki, K., *Agr. Biol. Chem.*, **39**, 1501

(1975)
7) Zacharius, R.M., Zell, T.E., Morrison, J.H., and Woodlock, J.J., *Anal. Biochem.*, **30**, 148 (1969)
8) Thanh, V.H., Okubo, K., and Shibasaki, K., *Plant Physiol.*, **56**, 19 (1975)
9) Catsimpoolas, N., Ekenstam, C., Rogers, D.A., and Meyer, E.W., *Biochim. Biophys. Acta*, **168**, 122 (1968)
10) Koshiyama, I., *Agr. Biol. Chem.*, **35**, 385 (1971)
11) Thanh, V.H., Master thesis, Tohoku University, Faculty of Agriculture (1974)
12) Poole, T., Leach, B.S., and Fish, W.W., *Anal. Biochem.*, **60**, 596 (1974)