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The Relationship between the Ultracentrifugal Fraction and the Gel Electrophoretical Bands of Soybean Proteins

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Summary

Water extracted proteins, main components C and D, isolated from defatted soybean meal, were analyzed by gel electrophoresis of starch and acrylamide and by ultracentrifugal analysis in systems without and with urea, respectively. It was revealed that, in system without urea, the main components C and D of soybean proteins were associated in form and corresponded mainly to 0.21 and 0.04 Rm on acrylamide gel electrophoretical pattern, and to the 11 and 7 S on ultracentrifugal pattern. Also the dissociated form of the C and D component corresponded to the B and B' bands on starch gel and to the bands in the region of 0.69–0.52 and 0.40–0.36 Rm on the acrylamide gel, respectively. In the system with urea, the C component was separated into acidic bands α, β, γ and several basic bands on starch gel, to 5 acidic and several basic bands on acrylamide gel, and shown to a single peak between 1 and 2 S on the ultracentrifugal pattern. The D component corresponded to a single band δ on the starch and the acrylamide gel and to a sigle peak between 1 and 2 S on ultracentrifugal pattern. And also the quarternary structure of the main component was discussed.

Gel electrophoresis has been a valuable tool in the characterization of a number of complex protein systems, but was applied to soybean proteins only recently[1-3].

In the ultracentrifugal studies[4-5], it has been revealed that soybean proteins consist of at last four components having $S_{20,w}$ of 2,7,11 and 15 S, respectively, of which the 7 and 11 S components constitute the major parts. More detailed studies[6,7] by ultracentrifugation revealed that the breakdown of the 7 and 11 S components into subunits in the 2 S region occurs at high and low pH, in the presence of urea, guanidine and anionic detergents.

In our ealier studies[1,8,9] by the starch gel electrophoresis, it has been revealed that the soybean proteins consist of components extending from bands A to E in a system whithout urea and bands 1 to 14 in a system with urea. These bands C and D or 7 to 10 constituted the major parts. Also when Tombs[2] used

acrylamide gel in the absence of urea to analyze the components of protein bodies, two major bands were observed and believed to glycinin. Puski and Melnychn[3] developed an alkaline and acidic buffer with urea for starch gel electrophoresis of soybean globuline and observed 14 bands in alkaline buffer and 15 bands in acidic buffer.

The present paper deals with the relationship between the ultracentrifugal fraction and the gel electrophoretical bands of soybean proteins.

Experiment

Materials

Soybeans (species, Nemashirazu) harvested in 1966 grown at Iwanuma, Miyagi, Japan, were stored at 5°C until used. Defatted meal, water extracted proteins, C and D components were prepared as described previously[8]. The 2-mercaptoethanol was of Tokyo Kasei Products and was used to eliminate disulfide polymerization. The urea was of Koso Chemical Products, recrystallized from water and ethanol, and was used to unfold the protein components. To the 0.076 M tris-citrate buffer (pH 8.6) and to the phosphate buffer (pH 7.6, 0.0327 M K_2HPO_4 and 0.0026 M KH_2PO_4) were added a 0.02 M concentration of 2-mercaptoethanol as solvent.

Ultracentrifugal analysis

The ultacentrifugal analysis was made at 20°C with a model UCA-1 Hitachi ultracentrifuge and all photographs of the schlieren patterns were taken after 36 min centrifugation at 55,430 r.p.m.. A normal cell was used in the system without urea (0.076 M tris-citrate buffer, pH 8.6, containing 0.02 M 2-mercaptoethanol) and a boundary synthetic cell in the system with urea (0.076 M tris-citrate buffer, pH 8.6, containing 0.02 M 2-mercaptoethanol and 8 M urea)

Starch gel electrophoresis

Starch gel electrophoresis was made at 5°C as described previously[1,2].

Acrylamide gel electrophoresis

The apparatus and the plastic tray were similar to those used in gel electrophoresis. In the system without urea, the acrylamide gel had a composition of 7.0% acrylamide monomer and 0.02 M 2-mercaptoethanol in a 0.076 M tris-citrate buffer (pH 8.6) and in the system with urea a composition of 9.0% acrylamide monomer, 0.02 M 2-mercaptoethanol and 6 M urea in the same buffer. The electrophoresis was carried out by using 175 V potential difference (8–10 mA) for 14–16 hrs..

Result

Starch Gel Electrophoretical Analyses

In our ealier studies[1,2] by starch gel electrophoresis, it has been revealed that the water extracted proteins consist of components corresponding to bands

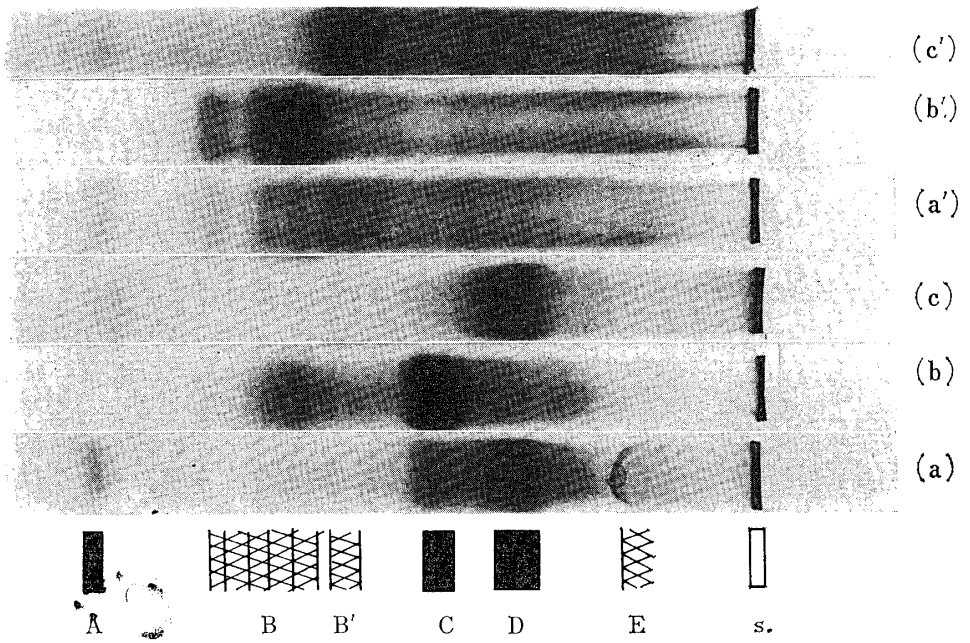


FIG. 1. Starch Gel Electrophoretical Patterns of Water-extracted Proteins (a), C (b' b') and D (c, c') in System without Urea
b', c': urea treated proteins. The preparation of urea treated proteins: dissolved in 0.07M tris-citrate buffer (pH 8.6) containing 8M urea and 0.02M 2-mercaptoethanol and stood at 30°C for 60 min.

A to E a system without urea or to bands 1 to 10 in a system with urea. Each component correspondig to the band C and D has been fractionated imperfectly by means of dialysis[4] and by gel filtration with Sephadex G-200, resulting in a higher purification of the C and D components[5].

In Fig. 1 are shown the starch gel electrophoretical patterns of those fractions of soybean proteins. From those results, it is appearant that the C component corresponds to band B in the system without urea and the bands 7,8,9 and the bands migrated nearer to the slot in system with urea and that the D component corresponds to band B' in the system without urea and to band 10 in the system with urea.

Acrylamide Gel Electrophoretical Analyses

An even greater complexity of soybean proteins was indicated by the acrylamide gel electrophoresis in the systems without or with urea (Fig. 3,4). A relative mobility (Rm) scale arbitrarily set at 1 at the fast-moving front is included in Fig. 2. In the system withour urea, at least seven bands migrated toward the positive electrode, of which the band at Rm value 0.04 is the most prominent and the band at 0.21 is next in intensity. Also two minor bands between the main bands, 0.14 and 0.10 were detected. In the system with urea, at least eleven bands migrated toward the positive electrode and several bands migrated nearer to the slot, of which the bands at Rm value 0.31 is most prominent and the bands

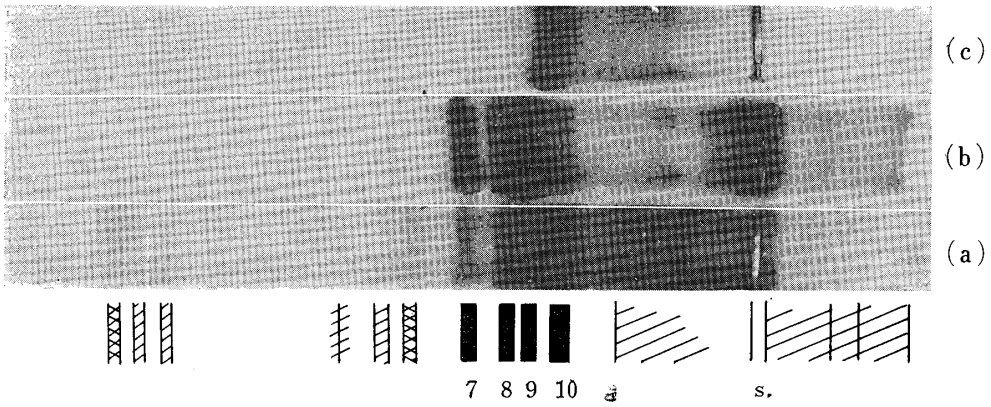


FIG. 2. Starch Gel Electrophoretcal Patterns of Waterr-extracted Proteins (a), C (b) and D (c) Component in System with Urea

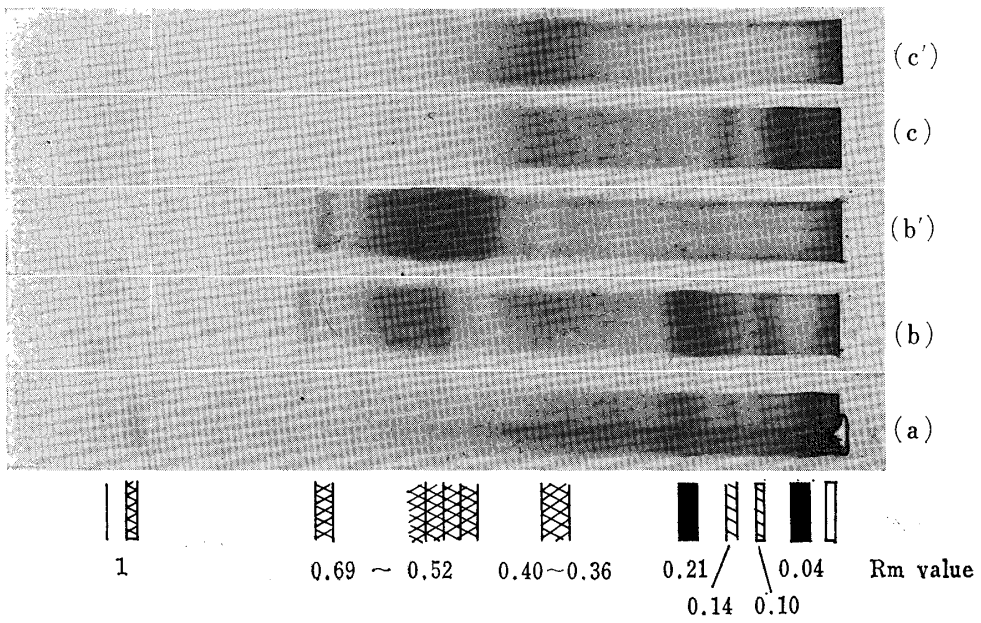


FIG. 3. Acrylamide Gel Electrophoretical Patterns of Water-extracted Proteins (a), C (b, b') and D (c, c') Component in System without Urea
b', c': urea treatted proteins

in the region of 0.54–0.35 are next in intensity.

The C component corresponded mainly to the band 0.21 and the bands in the region of 0.69–0.52, of which the bands in the region of 0.69–0.52 are a dissociated form of the C component because the band at 0.21 were changed into bands in the region of 0.69–0.52 by urea. The minor band at 0.10 detected in the C component preparation may be a contaminant and correspond to the 7 S component which does not dimerize when the ionic strength is lowered to 0.1[10], because in the ultracentrifugal pattern of the C component preparation a slight contaminant of the 7 S component was detected (Fig. 5).

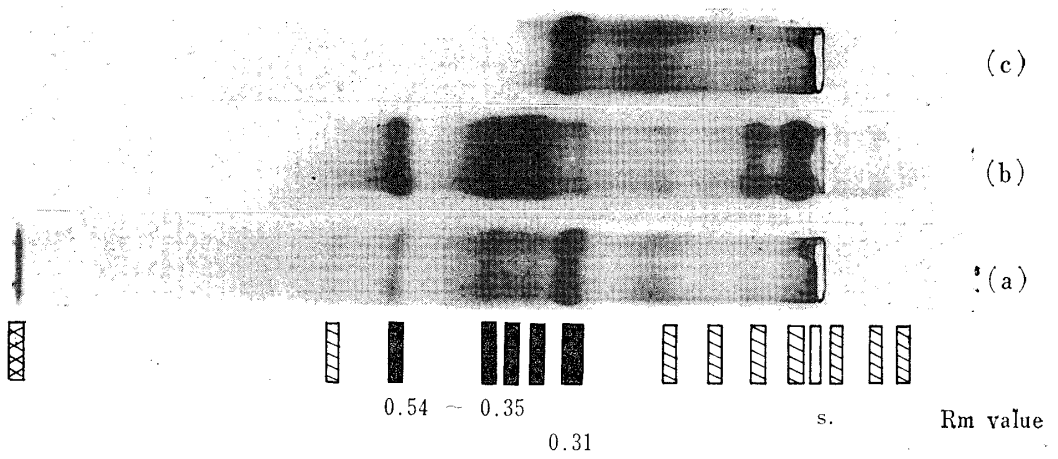
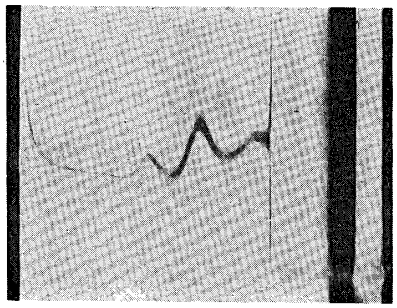


FIG. 4. Acrylamide Gel Electrophoretical Patterns of Water-extracted Proteins (a), C (b) and D (c) Component in system with urea

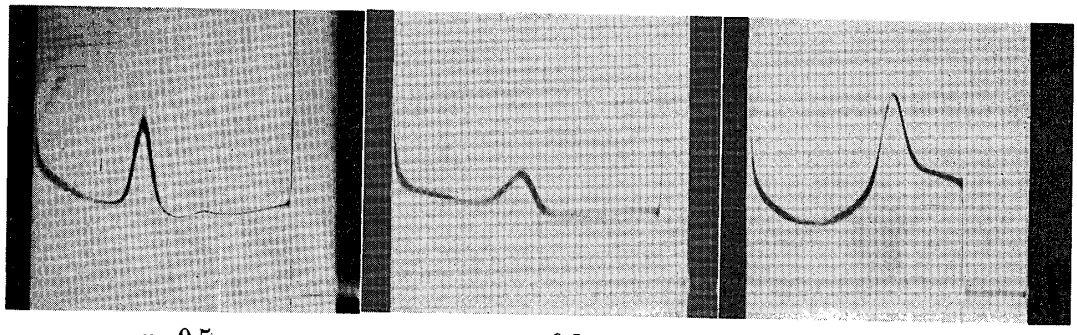
The D component corresponded mainly to the bands at 0.04 and in the region of 0.40–0.36, of which the band at 0.04 are an associated form of the D component, because band 0.04 was changed into a band in the region of 0.40–0.36 by urea. From those results it is appearant that the band at 0.21 corresponds to band C in the starch gel electrophoretical pattern and the bands in the region of 0.96–0.52 to band B. The Rm value of the D component in the acrylamide gel electrophortical patterns slightly different from that of water extracted proteins, which suggests that the charge of the D component changed slightly in the preparation. Minor bands at 0.14 detected in the D component may correspond to the 7 S component which dimerize when the ionic strength is lowered to 0.1[10], because in the ultracentrifugal pattern of the D component preparation the 7 S component was not detected at pH 7.6, 0.1 ionic strength.

In Fig. 4 are shown the acrylamide gel electrophoretical patterns of the system with urea of water extracted proteins, the C and D components. The C component corresponded with the four bands in the region of 0.54–0.35 and the several bands migrated nearer to the slot. The D component corresponded mainly with the band at 0.31.

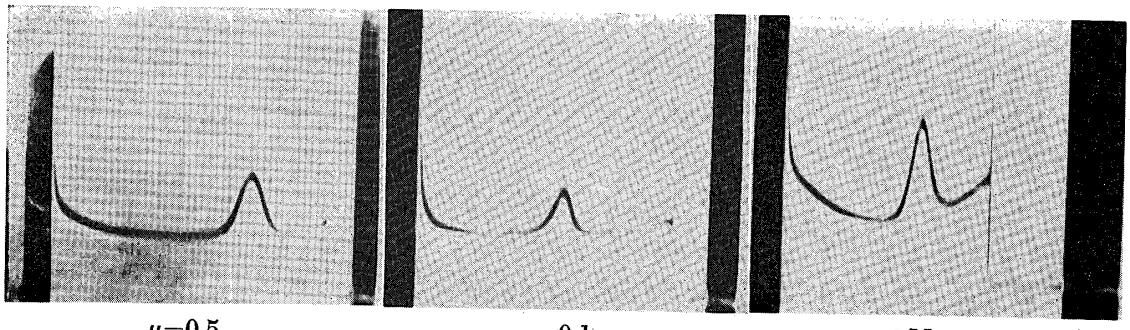
In our ealier studies[5], the subunits of the C component which corresponded with bands 7,8,9 and the bands which migrated nearer to the slot on the starch gel electrophoretical pattern in the system with urea, could be fractionated by column chromatography on DEAE-cellulose with urea. Those subunits are distinct from each other in physicochemical properties, amino acid composition and N-terminal amino acid composition[11]. On the acrylamide gel electrophoretical pattern of those subunits in the system with urea, bands 7, 8, 9 and the alkaline subunits correspond to the bands at 0.54, in the region of 0.42–0.39 and to the bands which had migrated nearer to the slot, respectively.



μ=0.5
Water-extracted proteins



μ=0.5 μ=0.1 8 M urea
C component



μ=0.5 μ=0.1 8 M urea
D component

FIG. 5. Ultracentrifugal Patterns of Water-extracted Proteins, C and D component using phosphate buffer, pH 7.6, (0.076 M K_2HPO_4, 0.0026 M KH_2PO_4 and 0.01 M 2-mercaptoethanol), ionic strength adjusted with sodium chloride, protein conc.: 0.7%, 36 min at 55,430 r.p.m., bar angle 60–70°, 20°C

Ultracentrigual Analyses

The ultracentrifugal studies[4,5] revealed that soybean proteins consist of at least four components having a sedimentation constant of 2,7,11 and 15 S, respectively, among them the 7 and 11 S components constitute the major parts.

The ultracentrifugal patterns of water extracted proteins, the C and D component preparations are shown in Fig. 5. In the ultracentrifugal pattern of water extracted proteins, at least four peaks can be detected. The 7 S component is more abundant than the 11 S component in Japanese soybean and dimerize in ionic

strength of 0.1[6,11]. Therefore the component corresponding to those peaks are refered to as the 7 S and 11 S components, respectively.

Nearly all the ultracentrifugal patterns of the C and D component preparation indicate a single peak with the exception of a slight contaminant in the C component preparation. The boundaries to those peaks were calculated to have a sedimentation constant $(S_{20,w})\mu$ of 12.39 and 7.96 in $\mu=0.5$, and 12.31 and 9.56 in $\mu=0.1$, respectively. Therefore the C and D components correspond to the 11 and 7 S components, respectively, in the ultracentrifugal analysis.

More detailed studies by ultracentrifugation[7] indicated that the 7 and 11 S components are capable of dissociating into subunits in the region of 1–2 S in the presence of urea and detergent. The ultracentrifugal patterns of water extracted proteins, the C and D component preparations in 8 M urea show single peaks, respectively, having $S_{20,w}$ of 2.74, 2.37 and 2.47.

Discussion

The ultracentrifugal studies have led to a terminology originarilly introduced by Naismith[4] which is based on approximate sedimentation values of 2.7, 11 and 15 S. But soybean proteins are more complex than indicated by the ultracentrifugal analysis. Whole soybean proteins were separated into at last 14 bands by starch gel electrophoresis in a system with urea. However, the components of soybean proteins separated by the gel electrophoresis appear to have no obvious correlation with the ultracentrifugal components.

It has been reported[12] that the 11 S component has a molecular weight of about 350,000 and is capable of dissoiating into subunits under 8 M urea and 0.02 M 2-mercaptoethanol and contains at least twelve polypeptide chains, eight of which end in glycine, two in phenylalanine and two in leucine or isoleucine. But in our unpublished studies[10], the C component (11 S) has a molecular weight of about 360,000 under 8 M urea and 0.02 M 2-mercaptoethanol which indicates that the C component contans at least ten polypeptide chains, four of which end in glycine, three in in leucine or isoleucine, two in phenylalanine and one in tyrosine. Those subunits were separated by means of column chromatography on DEAE-cellulose contained urea and obviously different from each other in physiochemical properties, amino acid composition, and N-terminal amino acid composition.

For convenience in the acrylamide gel pattern of the C component in the system with urea (as shown in Fig. 6), the bands corresponding to the C component were named α, β, β' and basic. The subunits of the C component, corresponding to bands 7,8,9 and several bands nearer to the slot on starch gel electrophoretical pattern are fractionable by column chromatography on DEAE-cellulose. They correspond to α, β and β', γ and basic, respectively, on the acrylamide gel electrophoretical pattern in the system with urea. The dinitrophenylation of these

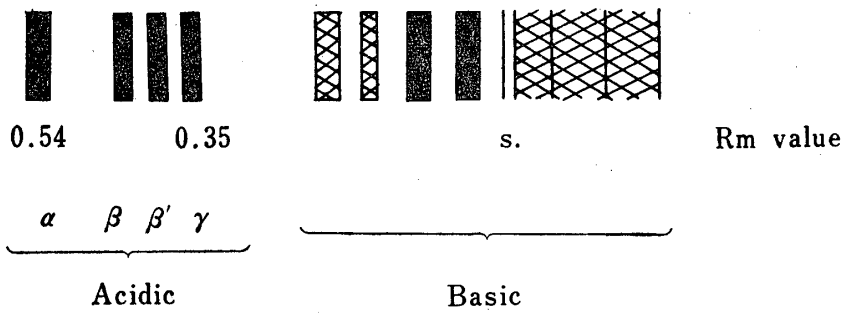


FIG. 6. Shematic Diagrams of Acrylamide Gel Electrophoretical Pattern of C component

TABLE 1.

Gel Electrophoresis			Ultra centrifuge	Molecular weight	Iso-electric point	N-terminal amino acid
Without urea	With urea					
Associated form	Dissociated form					
C			11S	350,000	4.7	Gly (4),Leu (Ilue) (3) Phe (2), Tyr (1)
	B Acidic	α_7	1~2S	30,000 ~ 35,000	4.8 ~ 5.2	Leu
		β_8				Leu (0.7), Tyr (0.3)
		γ_9				Phe
		Basic			8~9	Gly
D			7S	180,000	4.9	Asp (1), Ser (1), Glu (1), Gly (1), Ala (1), Try (1), Phe (1), Val (1), Leu (2)
	B'	d_{10}	1~2S	35,000	5.3	

fractions showed α a, mixture of β and β', γ and basic contained leucine or isoleucine, phenylalanine and tyrosine, leucine or isoleucine as N-terminal amino acid, respectively.

The D component, as well as the C component, are dissociable into subunits by urea, but the subunits could not be separated by gel electrophoresis, ultra-centrifugation and chromatography on DEAE-cellulose. However, this component contains eight kinds of amino acids as N-terminal. Therefore, those subunits, seem to have similar molecular weights and charge.

The discussin mentioned above was summarized in Table 1.

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