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Differences in Subunit Composition of Glycinin among Soybean Cultivars

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The subunit composition of glycinins isolated from the seeds of 18 cultivars of soybean (Japanese, U.S., Korean, and Chinese soybeans) were analyzed by electrophoreses on polyacrylamide gels under various conditions. From the analyses by either sodium dodecyl sulfate or alkaline urea gel electrophoresis, the glycinins of various cultivars could be classified into two groups, one of which contained an extraneous subunit protein, the most acidic one. When glycinins were analyzed by gel electrofocusing in the presence of urea and 2-mercaptoethanol, they could be classified into five groups according to the differing molecular charges of the subunits: group I contained seven acidics and eight basics; group II, seven acidics and seven basics; group III, six acidics and seven basics; group IV, six acidics and five basics; and group V, six acidics and three basics.

Grain legumes are potential sources of edible vegetable proteins for supplementing dietary needs. It is generally known that the major components of the seed storage proteins are responsible for contributing to the quality of foods made from these seeds, their flour and protein products, particularly the physical and nutritional properties. Not only are soybeans used for various kinds of traditional Japanese foods but also their protein products as well are used commercially as ingredients in foods. However, the lack of basic information about soybean protein components has hindered the full and effective utilization of the soybean and its protein products for food. The seed storage proteins of legumes contain legumin (referred to as the 11S component) which occurs in large amounts and appears to have a structure of $\alpha_6\beta_6$, in which α and β are acidic and basic subunit proteins, respectively (Derbyshire et al., 1976). Glycinin, one of the major components of the soybean storage protein, has been shown to be composed of three kinds each of acidic and basic subunits (Catsimpoolas, 1969). Kitamura et al. (1976) have reported the isolation of four kinds each of acidic and basic subunits of glycinin and shown that the acidic and basic subunits are linked together in specific combinations through disulfide bridges with the resulting formation of intermediary subunits. We have been studying the subunit structures of the major components of legume seed storage proteins. Previously, we found that the subunit composition of glycinin was different in Japanese soybean cultivars of Tsuru-no-ko and Raiden (Mori et al., 1979) and also that the subunit composition of legumin differed in broad bean cultivars as to the number and proportion of the subunit proteins (Utsumi et al., 1980). In this paper we report our investigation of the subunit composition of glycinin from a wide range of cultivars of soybean seeds applying gel electrophoresis under various conditions.

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Table I. List of Seed Samples Examined with Country of Origin

country	cultivar	sample no.
Japan	Tokachi Nagaha	1
	Shiro Tsuru-no-ko	2
	Rikuu No. 20	3
	Raiden	4
	Goyo Daizu	5
	Sakagami No. 2	6
	Iyo Daizu	7
	Matsuura	8
United States	Hill	9
	Hark	10
	Corsoy	11
	York	12
	Dare	13
	Ford	14
China	Bai-hua-zuo-zi	15
	Tianjin-dachingdou	16
Korea	Kinzu	17
	Huk-tae	18

MATERIALS AND METHODS

Materials. The samples of soybean seeds (*Glycine max*) examined and their country of origin are listed in Table I. The cultivars were grown at some places in Japan in 1977 where the seeds of each cultivar normally developed and matured: sample no. 1 and 2 were grown at Hokkaido Central Agricultural Experimental Station; no. 3–5 and 15–18, Iwate University Agronomy Farm; no. 6, The University of Tokyo Agrobiology Farm; no. 7 and 8, Kyushu National Agricultural Experimental Station; no. 9–14, Tohoku National Agricultural Experimental Station. Urea purified for biochemical research was obtained from Nakarai Chemicals (Japan). Ampholine was purchased from LKB Co.

Preparation of Acetone Powder. The seeds of soybean cultivars were soaked overnight in distilled water at 4 °C. Cotyledons from which the germ had been removed were homogenized with 15 volumes (w/v) of 63 mM Tris-HCl buffer (pH 7.8) containing 10 mM 2-mercaptoethanol and allowed to stand for 1 h at 20 °C with gentle

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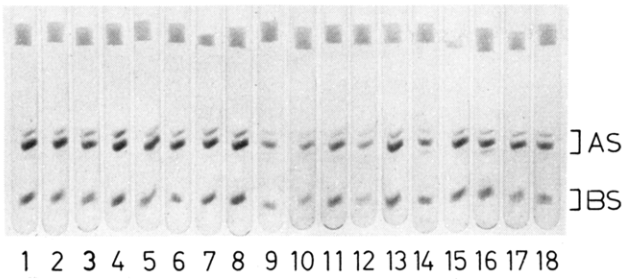

Figure 1. $NaDodSO_4$–polyacrylamide gel electrophoresis in the presence of 2-mercaptoethanol of glycinins from samples, no. 1–18, listed in Table I. 50 μg of each glycinin was used for electrophoresis.

stirring. The homogenate was filtered through gauze and then centrifuged at 9000 rpm for 15 min at 0 °C. Chilled acetone was added slowly to the supernatant to 60%. The precipitate was collected by centrifugation, washed once with chilled acetone, washed once with diethyl ether, dried, and stored in a desiccator at 4 °C until used.

Preparation of Purified Glycinin. The crude glycinin fraction was prepared from the acetone powder according to the method of Thanh et al. (1975). Chromatographic fractionation of the crude glycinin fraction was performed on a column of DEAE-Sephadex A-50 according to the method reported previously (Mori et al., 1978), except that elution was carried out in steps using 0.25, 0.275, and 0.35 M NaCl. The eluate between 0.275 and 0.35 M NaCl (glycinin fraction) was dialyzed against 0.035 M potassium phosphate buffer (pH 7.6) containing 10 mM 2-mercaptoethanol, 0.4 M NaCl, and 0.02% NaN_3 in the cold and then fractionated by centrifugation on a 10–30% (w/v) linear sucrose density gradient in the same buffer at 34000 rpm at 20 °C for 16 h in a Hitachi RPS 40T rotor as described previously (Mori and Utsumi, 1979). Of the three major peaks which appeared in the gradient, the third one (purified glycinin fraction) was subjected to further analyses.

Electrophoresis. Sodium dodecyl sulfate ($NaDodSO_4$) gel electrophoresis was performed according to the method of Laemmli (1970) at room temperature with 10% polyacrylamide gels in the presence or absence of 2-mercaptoethanol as described previously (Mori and Utsumi, 1979). Alkaline gel electrophoresis was performed with 7.5% polyacrylamide gels at 4 °C according to the method of Davis (1964) in 7 M urea in the presence or absence of 2-mercaptoethanol as described previously (Mori and Utsumi, 1979). Gel electrofocusing in the presence of 7 M urea and 2-mercaptoethanol was performed according to the method of Wrigley (1971) with a slight modification as described previously (Utsumi et al., 1980).

RESULTS

The purified glycinins from various cultivars were analyzed by electrophoresis on $NaDodSO_4$–polyacrylamide gel in the presence of 2-mercaptoethanol. As shown in Figure 1, all glycinins were composed of three major subunit proteins with molecular weights of 38000, 34800, and 19000–17900, data which were consistent with the results obtained earlier (Mori et al., 1979) when it was shown that the larger two subunits and the smaller one were acidic and basic subunits, respectively. When the glycinins were analyzed by $NaDodSO_4$ electrophoresis in the absence of 2-mercaptoethanol, two band patterns were observed (Figure 2). In one, only a band corresponding to the intermediary subunits (IS) appeared, while in the other, two more bands appeared besides the IS band. One of the extra bands corresponded to the acidic subunit with a

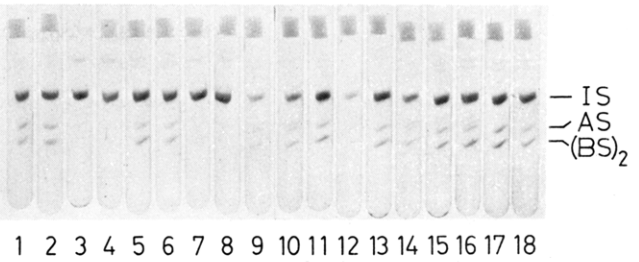

Figure 2. $NaDodSO_4$–polyacrylamide gel electrophoresis in the absence of 2-mercaptoethanol of glycinins from the same samples described in Figure 1.

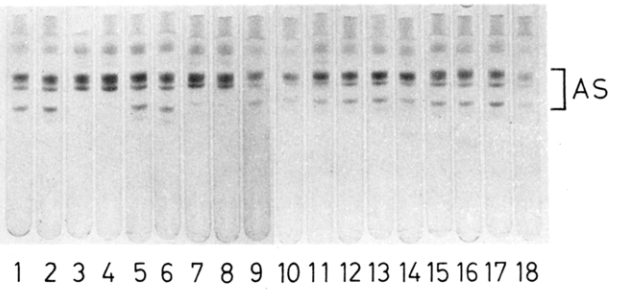

Figure 3. Alkaline urea–polyacrylamide gel electrophoresis in the presence of 2-mercaptoethanol of glycinins from the same samples described in Figure 1.

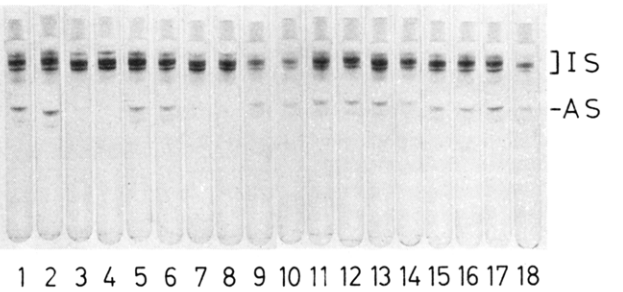

Figure 4. Alkaline urea–polyacrylamide gel electrophoresis in the absence of 2-mercaptoethanol of glycinins from the same samples described in Figure 1.

molecular weight of 34800, and the other band seemed to be a dimeric form of the basic subunits considering its migratory position. The self-association of the basic subunits of glycinin has been described in our previous paper (Mori et al., 1979).

Alkaline urea electrophoretic analysis of the glycinins in the presence of 2-mercaptoethanol produced two band patterns as shown in Figure 3. One group may be distinguished from the other by the presence of a band of the most acidic subunit. Similarly two band patterns were observed when the glycinins were analyzed in the absence of 2-mercaptoethanol (Figure 4). One group had only the IS band, while the other group had an additional band corresponding to the most acidic subunit. Identification of the bands in the gels under various electrophoretic conditions was made by comparing them with our previous results (Mori et al., 1979).

These results indicate that the glycinins of various cultivars could be classified into two groups, one which contained an extra subunit protein (the most acidic subunit). This acidic subunit appears to be linked to the basic subunit to form an intermediary subunit, not through a disulfide bridge but through some other interaction which can be disrupted by such denaturants and $NaDodSO_4$ and urea. Although the existence of such an intermediary subunit is rather unexpected, it may be a constituent of glycinin, since the total amount of the most acidic subunit and the basic subunit, both of which constitute the in-

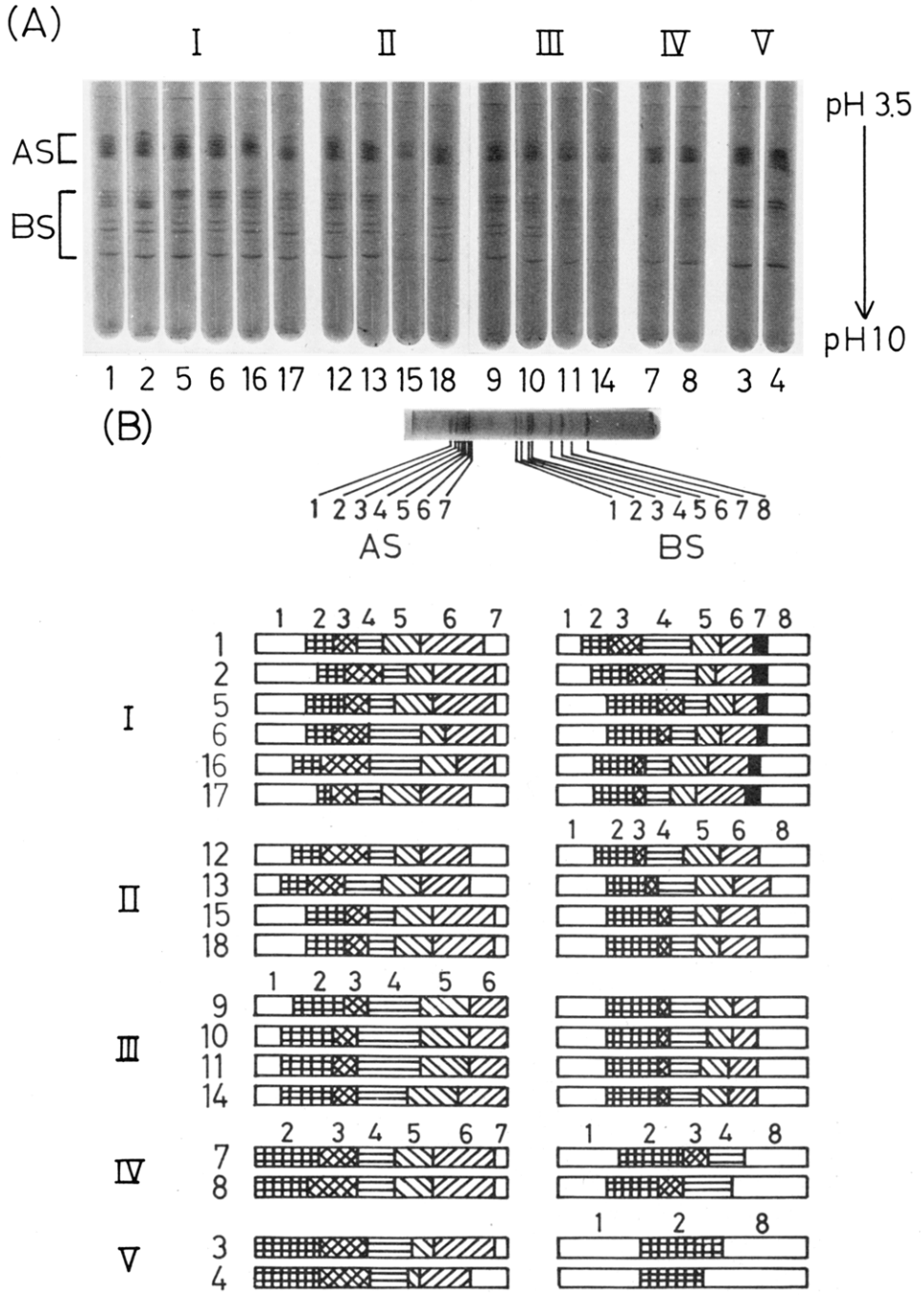

Figure 5. Gel electrofocusing of glycinins from the same samples as described in Figure 1 except that 100 μg of each glycinin was used (A). The relative proportion of the acidic and the basic subunits from each cultivar was calculated from densitometric scanning of the gels and is represented schematically (B).

termediary subunit, is comparable to that of the other subunits (Figures 2–4). Recently, Moreira et al. (1979) reported the existence of an acidic subunit with a molecular weight of ~10 000 in glycinins from CX635-1-1-1 and so did Kitamura et al. (1980) from Norin No. 1 and Amsoy. However, we did not observe such an acidic subunit in the $NaDodSO_4$ electrophoretic analysis under the conditions used here.

In order to obtain further details about the subunit compositions of glycinins from various soybean cultivars, we analyzed glycinins by polyacrylamide gel electrofocusing in the presence of urea and 2-mercaptoethanol. As shown in Figure 5, the subunits of glycinins separated into two zones. One shifted to the acidic region and the other shifted to the basic region. The numbers of the acidic and basic subunits differed among the cultivars. The patterns could be sorted into three groups for acidic subunits and four groups for basic subunits. For both acidic and basic subunits, the subunit compositions of glycinins could be classified into five groups: group I contained seven acidics and eight basics; group II, seven acidics and seven basics; group III, six acidics and seven basics; group IV, six acidics and five basics; group V, six acidics and three basics (Figure 5). The amount ratio of the subunit proteins was not unity in most of the cultivars, while it was roughly similar for cultivars in the same group. However, it is not clear whether the amount ratio of the subunit proteins in each cultivar is invariant or if they differ due to variation in physiological and environmental conditions.

Since no significant band between the acidic and basic regions in electrofocusing and no minor bands in $NaDodSO_4$ and alkaline urea electrophoreses were present, the

purities of the glycinin preparations used here seem to be sufficient to clarify their subunit composition in detail.

DISCUSSION

The subunit composition of legumin among cultivars has been investigated by Harada (1972) and Kitamura et al. (1980) for glycinin, by Tombs (1965) for groundnut, by Blagrove and Gillespie (1978) for lupin, and by Thomson and Schroeder (1978) and Casey (1979) for pea. However, their research has not indicated the number of the constituent subunits. In a previous paper, we studied the subunit compostion of legumins of broad bean seeds and found that the number and proportion of the subunits were different for the cultivars and that their band patterns could be arranged into three groups corresponding to the size of the seeds examined, i.e., small-, medium-, and large-sized groups (Utsumi et al., 1980). However, the band patterns of glycinin could not be grouped according to the size of the seeds unlike the case of broad bean legumin. Since the present study on soybean cultivars is not exhaustive, other types of subunit composition of glycinins may exist in soybean cultivars different from those described above. At any rate, our observations (Figure 5) support those of others as to the diversity of subunit composition and also suggest the heterogeneity of glycinin molecular species, as has already been postulated for peanut arachin (Tombs, 1965; Tombs and Lowe, 1967), pea legumin (Thomson et al., 1978), and broad bean legumin (Utsumi and Mori, 1980).

The differences in the subunit composition of glutenin among wheat cultivars and their relation to the bread-making quality of the flours have been studied by several people (Huebner, 1970; Orth and Bushuk, 1973; Bietz et al., 1975; Butaki and Dronzek, 1979). Although differences in the subunit composition of glutenin were observed in cultivars, no significant correlation with bread-making quality could be made. However, Payne et al. (1979) have recently found that the presence of a subunit of glutenin, whose molecular weight is ~145 000, correlated with bread-making quality. On the other hand, it has been reported that the quality of tofu (one of the Japanese traditional foods made from soybeans) differs according to the cultivars used (Smith et al., 1960). Saio et al. (1969) found the proportion of 7S and 11S globulins to be responsible for the differences in the physical properties of tofu gel among soybean cultivars. Thus, it seems likely that the subunit composition of glycinin is related to the physical properties of foods made from soybeans or their isolated proteins. From such a viewpoint we intend to study the relationship between the subunit compositions of glycinins and the exhibition of their functional properties such as gelation, emulsification, and so forth in the food system.

Moreira et al. (1979) have recently reported that the sulfur amino acids in glycinin from soybean cultivar CX635-1-1-1 are not distributed evenly among the various subunits, of which six acidic and four basic subunits have been distinguished. Their observation seems to have significance in efforts to improve soybean quality genetically with respect to the sulfur amino acid level. As described above, we could distinguish many more subunits than they did; e.g., seven acidic and eight basic subunits of glycinin existed in group I. Therefore, analyses of the amino acid compostion of those subunits as well as among the cultivars may provide further information about the uneven distribution of the sulfur amino acids in glycinins among the subunits for the purpose of soybean breeding.

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