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HETEROGENEITY OF SOYBEAN GLYCININ

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Key Word Index—*Glycine max*; Leguminosae; soybean; heterogeneity; glycinin.

Abstract—Glycinin from soybean var. Tsuru-no-ko was fractionated on a DEAE-Sephadex A-50 column and the molecular species were investigated by electrophoretic methods. The results obtained indicated the heterogeneity of glycinin molecular species. Four molecular species of apparent MWs 340000, 345000, 360000 and 375000 were detected by gradient gel electrophoresis. A similar heterogeneity was observed in the half-molecule of glycinin, that is, three molecular species of apparent MWs 199000, 211000 and 222000 were detected. Other cultivars which have different subunit compositions from that of Tsuru-no-ko also exhibit a similar heterogeneity of glycinin molecular species. These results together with the evidence obtained in our laboratory on broad bean legumin and by others on some seed globulins suggest that heterogeneity is an inherent property of the major storage proteins of legume seeds.

INTRODUCTION

Glycinin, an 11S globulin, is one of the most predominant components of soybean storage proteins. The structure of glycinin has been well investigated [1–10]: several models of the glycinin molecule have been proposed [1, 7, 8] and NH_2-terminal amino acid sequences of glycinin subunits have recently been presented [9, 10]. In these studies, glycinin was considered as a homogeneous protein with respect to molecular species. However, recently, heterogeneities have been found in 11S globulins from ground nut [11] and pea [12], and 7S globulins from soybean [13], pea [12] and lupin [14]. Previously, we demonstrated that legumin, an 11S globulin from broad bean, consists of four molecular species of different MWs [15]. These data suggest that heterogeneity is an inherent property of the major storage proteins of legume seeds in general.

In the present study, the heterogeneity of glycinin from soybean was investigated by the same methods as those used to study the heterogeneity of legumin from broad bean [15]. The results obtained demonstrate that glycinin exhibits a heterogeneity of molecular species from the standpoint of molecular size.

RESULTS AND DISCUSSION

Heterogeneity of the glycinin from soybean var. Tsuru-no-ko

Glycinin-rich fractions obtained by the methods of Thanh *et al.* [16] were applied to a DEAE-Sephadex A-50 column. The chromatogram of the glycinin from Tsuru-no-ko is shown in Fig. 1. The unbound fraction may be derived from the 7S component which contaminated the glycinin-rich fraction (see Fig. 3, gel 2). Glycinin eluted as

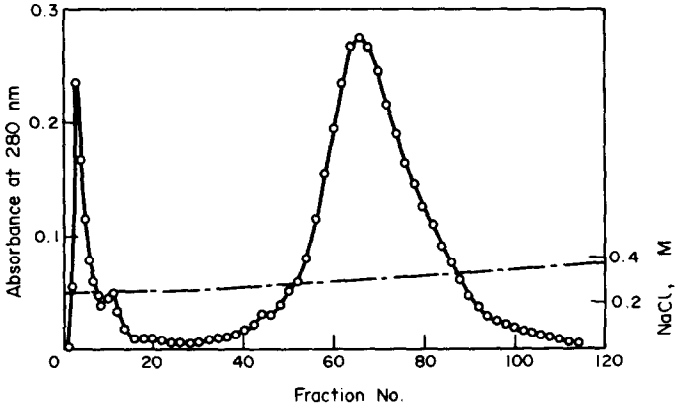

Fig. 1. DEAE-Sephadex A-50 column chromatography of glycinin-rich fraction. Glycinin (60 mg) was equilibrated with 35 mM KPi buffer (pH 7.6) containing 0.25 M NaCl and 0.02% NaN_3 and then applied on the column (1.5 × 17 cm) which had been equilibrated with the buffer at 4°. The column was washed with the buffer. Development: 600 ml buffer, 0.25 → 0.45 M NaCl; flow rate, 20 ml/hr; fraction volume, 5 ml. O——O, A_{280nm}; – – –, [NaCl].

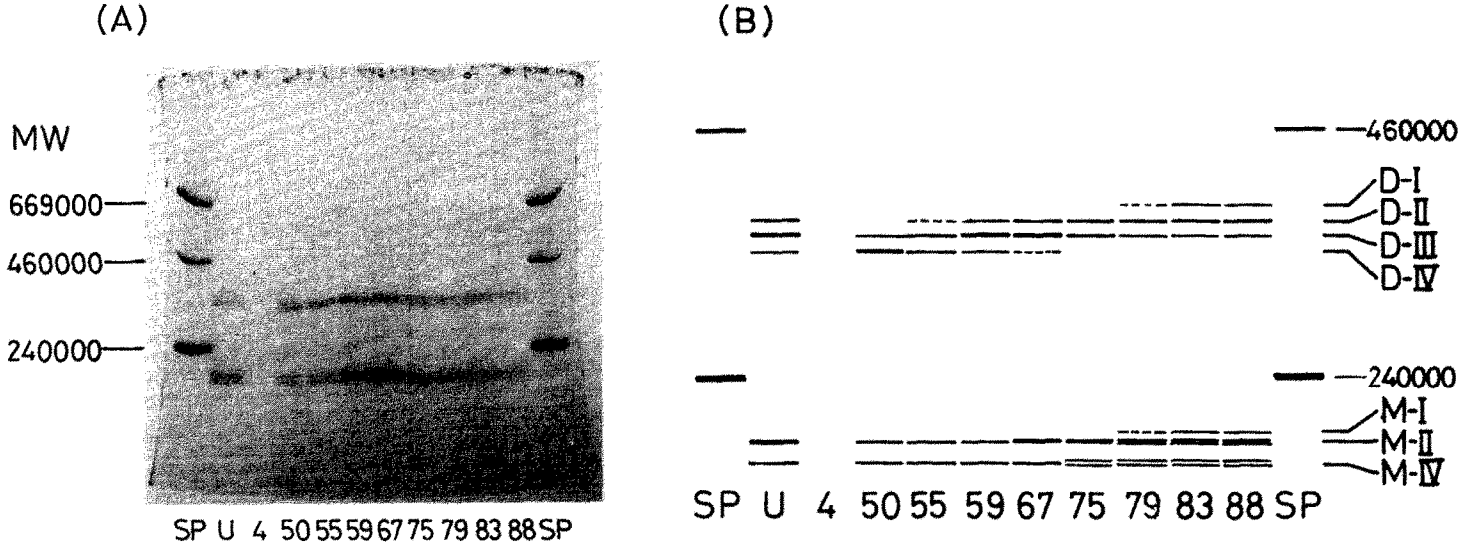

Fig. 2. Polyacrylamide gradient gel electrophoresis of glycinin fraction. (A) 20 μg protein of each fraction equilibrated with 35 mM KPi buffer (pH 7.6) containing 0.2 M NaCl and 10 mM 2-mercaptoethanol was applied on the gel (see [15] for details). SP and U refer to standard proteins (thyroglobulin, 669000; ferritin, 460000; catalase, 240000) and the unfractionated sample, respectively. The numbers under the gels are the fraction numbers of DEAE-Sephadex A-50 column chromatography. Migration is from top to bottom. (B) Diagrammatic representation of (A).

a single peak which was comparatively sharper than that of legumin from broad bean [15].

The fractions from the column were analysed by polyacrylamide gradient gel electrophoresis (4–30%). The electrophoretic patterns are shown in Fig. 2. Two zones were separated in all samples, the faster and slower groups of bands being monomers (half-molecules of glycinin) and dimers (glycinin), respectively. The monomer bands may be derived from the dissociation of the glycinin molecule during the electrophoresis, because the glycinin molecule has been shown to undergo reversible dissociation to a 7S monomer [17], although it is unclear whether it is intact or not. In fact, a similar monomer band was observed on PAGE by Kitamura *et al.* [18]. On the other hand, we did not observe such behaviour in the case of broad bean legumin [15], which does not undergo association–dissociation reactions with changes in ionic strength in the normal pH range [19, 20]. The unfractionated glycinin gave three bands with apparent MWs 360000 (D-II), 345000 (D-III) and 340000 (D-IV), of which the proportion of the middle band was higher than that of others (D-III > D-II, D-IV). Fractions 50, 59–67, 75 and 79–88 gave two (D-IV > D-III), three (D-III > D-II, D-IV), two (D-II = D-III) and three bands (D-II > D-I (MW 375000), D-III), respectively. The band of the largest molecular species (D-I) was not observed in the unfractionated glycinin. This may be due to the low content of this molecular species. The results thus obtained indicate the presence of four molecular species of glycinin from the standpoint of molecular size. However, when the fractions were subjected to PAGE a similar broad band was observed in every fractions, cf. plural bands with different migration rates given by the broad bean legumin fractions [15]. This indicates that, in contrast to broad bean legumin [15], the glycinin molecular species have similar molecular charges, and that many more molecular species may exist in glycinin than in legumin.

The unfractionated glycinin gave two monomer bands with apparent MWs 211000 (M-II) and 199000 (M-IV), of which the proportion of M-II was higher than M-IV. Fractions 50–67 gave the same monomer bands as those of the unfractionated glycinin. The slower eluting fractions contained more M-II. Considering the gel electrophoretic patterns of the monomers and dimers and their apparent MWs in fractions 50–67 (Fig. 2), the dimers D-II and D-IV may be assumed to be homodimers composed of the monomers M-II and M-IV, respectively. Similarly, the dimer D-III may be assumed to be a heterodimer made up of monomers M-II and M-IV. Since fractions 79–88 gave another monomer band of MW 222000 (M-I) accompanying the appearance of D-I, it may be assumed that M-I is one of the monomer components of D-I. M-IV, observed in fractions 50–67, was split into two bands in the fractions eluted after fraction 75. Similarly M-II was split into two bands in the fractions after fraction 80. This microheterogeneity of the monomer in the fractions after 75 or 80 may be partly responsible for the heterogeneity of the dimer. There was good correlation in the number of bands between the monomer and the dimer in fractions 55–67, while this was not the case in the fractions after fraction 75.

The fractions were analysed by SDS-PAGE in order to elucidate their subunit compositions. The electrophoretic patterns are shown in Fig. 3. All the fractions gave four bands, of which two were acidic with MWs 38000 and 34800, and another two were basic with MWs 19000 and 18300 [21]. The ratio of basic subunits was different among the fractions. Thus, the more slowly glycinin eluted, the higher the content of the subunit of MW 19000 and the lower the content of the subunit of MW 18300. The ratio of acidic subunits was almost similar among the fractions.

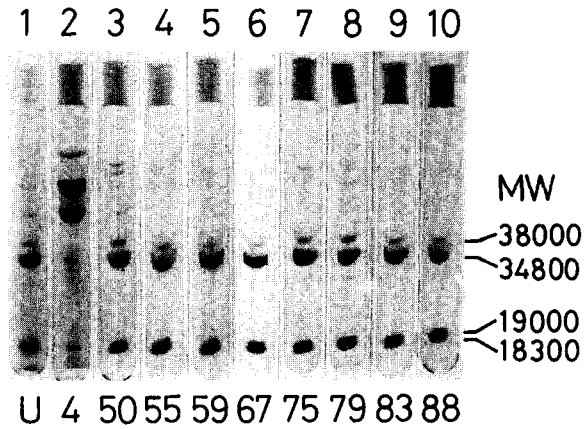

Fig. 3. SDS-PAGE of glycinin fraction. 30 μg protein of each fraction was dialysed against 62.5 mM Tris–HCl buffer (pH 6.8) containing 1% SDS and 0.2 M 2-mercaptoethanol for 20 hr at room temperature and then electrophoresed as described in the Experimental. U refers to the unfractionated sample. The numbers under the gels are the fraction numbers of DEAE-Sephadex A-50 column. Migration is from top to bottom.

The difference in the MWs of the basic subunits does not seem to account for the differences in the MWs of the dimers and those of the monomers. However, it has been shown that the acidic subunit of MW 34800 and the basic subunits are heterogeneous from the standpoint of their molecular charges [1, 6, 10, 21, 22], and that glycinin has intermediary subunits ($\alpha\beta$), disulfide-bonded acidic (α) and basic (β) subunits, and is composed of 6 ($\alpha\beta$) [6, 8, 21]. Therefore, there may be some kinds of intermediary subunits composed of subunits which are identical in their MWs but are different in their molecular charges. Thus, the number and the position of disulfide bonds contributing to the formation of intermediary subunits may be different. These differences should effect the compactness of their structure, which in its turn may influence the apparent MW of each molecular species of glycinin. We have reported previously a similar phenomenon for the intermediary subunits of broad bean legumin [15].

From analysis of the subunit composition of glycinin fractions eluted from the column by gel electrofocusing in the presence of urea and 2-mercaptoethanol, we found differences in the subunit composition from the standpoint of their charges among the fractions (data not shown). The exact subunit composition of each molecular species is currently being investigated by considering the results described in this paper.

Heterogeneity of glycinin in other cultivars

To elucidate whether each glycinin from a wide range of soybean cultivars exhibits the heterogeneity of molecular species, the glycinins from various soybean cultivars, i.e. Shiro-tsuru-no-ko, Tianjin-dachingdou, York, Ford, Iyo-

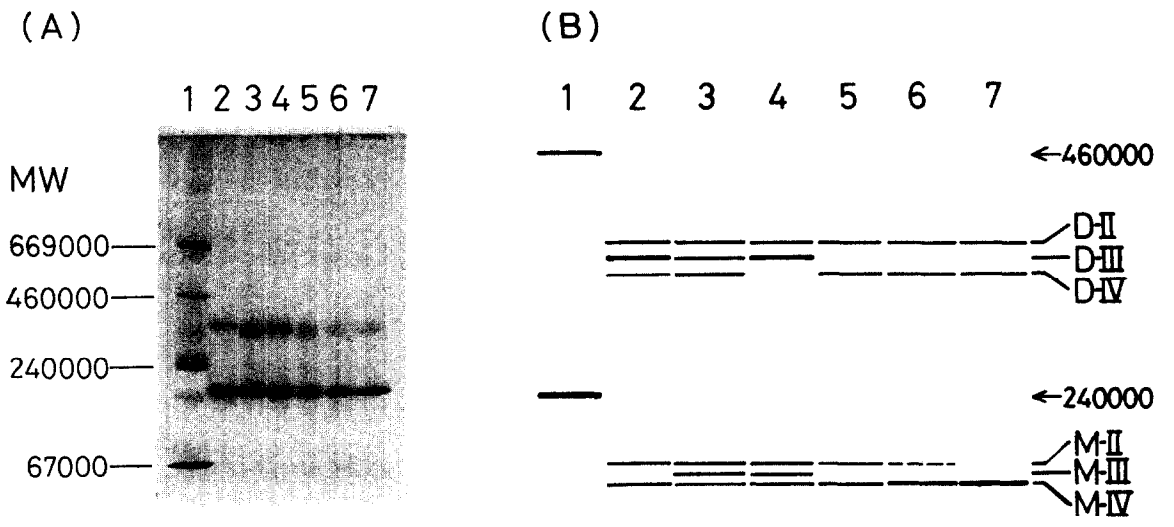

Fig. 4. Polyacrylamide gradient gel electrophoresis of glycinins from various soybean cultivars. (A) 20 μg protein of each cultivar was electrophoresed as described in Fig. 2. Migration is from top to bottom. Column 1, standard proteins (thyroglobulin, ferritin, catalase and bovine serum albumin, 67000); column 2, Shiro-tsuru-no-ko; column 3, Tianjin-dachingdou; column 4, York; column 5, Ford; column 6, Iyo-daizu; column 7, Raiden. (B) Diagrammatic representation of (A).

daizu and Raiden, were analysed by polyacrylamide gradient gel electrophoresis. The glycinins of these soybean cultivars have been shown to be different in their subunit compositions. Thus Shiro-tsuru-no-ko and Tianjin-dachingdou are composed of 7 acidics and 8 basics; York, 7 acidics and 7 basics; Ford, 6 acidics and 7 basics; Iyo-daizu, 6 acidics and 5 basics; Raiden, 6 acidics and 3 basics from the standpoint of the molecular charges of subunits [22]. As shown in Fig. 4, the glycinin from Shiro-tsuru-no-ko gave three dimer bands, D-II, D-III and D-IV, and two monomer bands, M-II and M-IV; Tianjin-dachingdou, three dimer bands, D-II, D-III and D-IV, and three monomer bands, M-II, M-III (MW 205000) and M-IV; York, two dimer bands, D-II and D-III, and three monomer bands, M-II, M-III and M-IV; Ford, two dimer bands, D-II and D-IV, and two monomer bands, M-II and M-IV; Iyo-daizu, two dimer bands, D-II and D-IV, and two monomer bands, M-II and M-IV; Raiden, two dimer bands, D-II and D-IV, and one monomer band, M-IV. The results show that the numbers of glycinin molecular species are different among the cultivars. The extent of the heterogeneity of the glycinin seems to correlate with the diversity of the subunit composition of the glycinin.

EXPERIMENTAL

Purification of glycinin. The seeds of soybean (Japanese var. Tsuru-no-ko, Shiro-tsuru-no-ko, Iyo-daizu and Raiden, Chinese var. Tianjin-dachingdou, U.S.A. var. York and Ford) were soaked overnight in distilled H_2O at 4° and then dehulled. The wet dehulled soybean seeds (3 g) were homogenized in 45 ml 63 mM Tris–HCl buffer (pH 7.8) containing 10 mM 2-mercaptoethanol and stirred for 1 hr at 20°. The homogenate was filtered through gauze and the filtrate centrifuged at 9000 rpm for 15 min at 0°. Chilled Me_2CO was slowly added to the supernatant up to 60% saturation below 0°. The ppt. was collected by centrifugation at 8000 rpm for 15 min at −20°, washed with chilled Me_2CO and dried in a desiccator. The glycinin-rich fractions were prepared from the Me_2CO powders according to the method of ref. [16] and then were applied to DEAE-Sephadex A-50 columns as described previously [21]. The glycinin from Tsuru-no-ko was eluted by a NaCl gradient, while those from other cvs were eluted stepwise.

Electrophoretic methods. SDS-PAGE was performed by the method of ref. [23] as described previously [20]. Polyacrylamide gradient gel electrophoresis was performed according to the procedure described previously [15], except that the electrode buffer used was 50 mM Tris and 384 mM glycine (pH 8.3).

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