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SHORT REPORTS

POLYMORPHISM OF GLYCININ IN SOYBEAN SEEDS

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

INTRODUCTION

Glycinin, a well-characterized major storage protein of soybean seeds is composed of acidic and basic subunits of MW 28 000–45 000 and 18 000–22 500, respectively [1–3]. Electron microscopy and X-ray scattering studies have revealed that these subunits are packed in two identical hexagons, placed one on the other [4]. However, some discrepancy exists concerning the number and MWs of subunits in the protein molecule. In order to resolve this, we have tried to compare the subunit composition of glycinin purified from 4 cvs of soybeans.

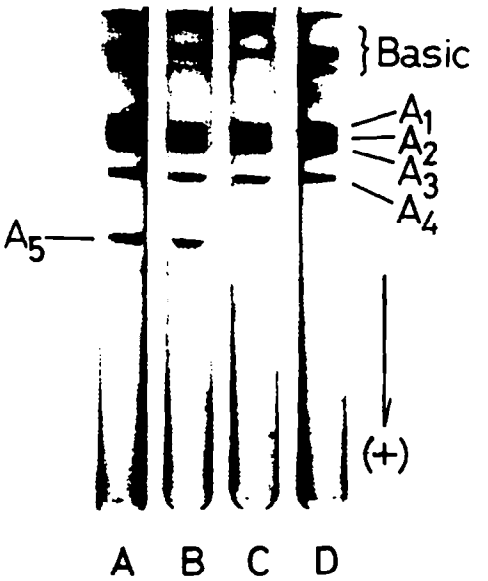

Fig. 1. Disc PAGE of RCAM-glycinin in the presence of 7 M urea from (A) cv Norin No. 1, (B) cv Amsoy, (C) cv Tokyo and (D) cv Raiden soybeans. Lyophilized RCAM-glycinin (1 mg) was dissolved in 1 ml of 50 mM Tris–HCl buffer (pH 8.6) containing 8 M urea. The protein samples (20 μl) were applied on top of the gel and layered with the electrode buffer containing 4 M urea. Electrophoresis was carried out with 6.5% (w/v) polyacrylamide (1:30 cross-linkage) in the separation gel with urea at pH 8.9 at a constant current of 1 mA per tube for *ca* 1.5 hr.

RESULTS AND DISCUSSION

Figure 1 shows disc polyacrylamide gel electrophoresis (PAGE) of *S*-carboxyamidemethylated (RCAM)-glycinin from the 4 cvs of soybeans in the presence of 7 M urea. Cv Tokyo RCAM-glycinin gave the 4 acidic subunit bands corresponding to the 4 acidic subunits A_1, A_2, A_3 and A_4, and the basic subunits of cv Raiden RCAM-glycinin [5]. On the other hand, in addition to these subunit bands, one major (designated as A_5 subunit in Fig. 1) and a few minor subunit bands were observed on the disc gels of RCAM-glycinin from cv Norin No. 1 and cv Amsoy soybeans.

Figure 2 shows SDS slab PAGE patterns of RCAM-glycinin from the 4 cvs of soybeans. The SDS slab PAGE used allowed high resolution of the subunits compared with that obtained by the ordinary SDS disc PAGE [1, 2]. In addition to the subunit bands of RCAM-glycinin from cv Raiden and cv Tokyo, a major and a minor subunit band were observed on the gels of RCAM-glycinin from cv Norin No. 1 and cv Amsoy. Preliminary investigation in our laboratory has revealed that an extra major subunit (A_5) on the disc gel with urea of RCAM-glycinin from cv Norin No. 1 and cv Amsoy corresponds to an extra major subunit on the SDS slab gel of RCAM-glycinin from the two cvs of soybeans.

Although each purified glycinin from cv Norin No. 1 and cv Amsoy was shown to be homogeneous by ultracentrifugal analysis, even so the extra subunit might be an impurity. In order to demonstrate that the extra subunit is part of the glycinin molecule, 2-D PAGE, in which the subunit is shown to behave as a true glycinin subunit, was performed. The purified cv Amsoy glycinin gave two components, a major one and a faster running component on the first dimensional gel of the Davis–Ornstein [6, 7] electrophoretic system. The faster running component showed almost the same electrophoretic pattern of subunit bands as that of the major component in the second dimension on two 2-D gels indicating that the faster running component is not an impurity, but the half molecule of glycinin [8]. The similar half-molecule band has been observed on the disc gel of cv Raiden glycinin [5]. Fig. 3(A, B) shows that the component in the first dimen-

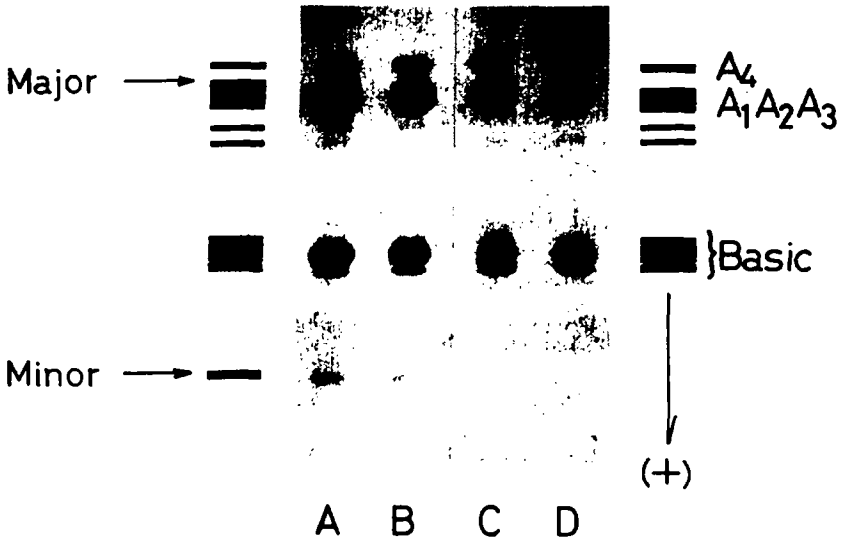

Fig. 2. SDS slab PAGE and schematic representation of the patterns of RCAM-glycinin from (A) cv Norin No. 1, (B) cv Amsoy, (C) cv Tokyo and (D) cv Raiden soybeans. Lyophilized RCAM-glycinin (1 mg) was dissolved in 1 ml of 50 mM Tris–HCl (pH 8.6) containing 0.2% SDS and 6 M urea. Protein samples (50 μl) were electrophoresed with a constant voltage of 120 V until the bromophenol blue marker reached the bottom of the gel (*ca* 7 hr). Arrows indicate the extra major and minor subunit bands which are not observed on the gels of RCAM-glycinin from cv Tokyo and cv Raiden.

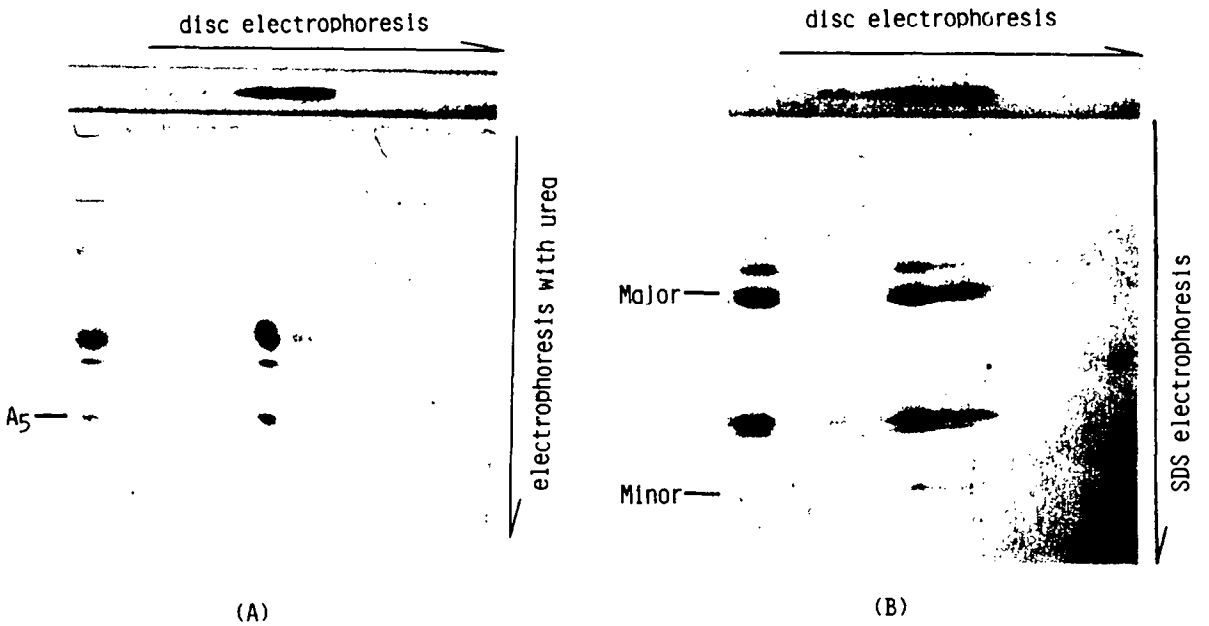

Fig. 3. 2-D PAGE of the purified cv Amsoy glycinin using the disc PAGE in the first dimension and (A) the slab gel PAGE with urea and (B) the SDS slab PAGE in the second dimension. Protein sample (10 μl, *ca* 0.2% in 35 mM potassium phosphate buffer, pH 7.6 containing 0.4 M NaCl and 0.01 M 2-mercaptoethanol) was electrophoresed with a constant voltage of 75 V for *ca* 4 hr in the first dimension. After incubating the disc gel in an excess amount of 8 M urea solution containing 0.1 M 2-mercaptoethanol or of 6 M urea solution containing 0.2% SDS and 0.1 M 2-mercaptoethanol for 30 min at 40°, the gel was layered on the second dimensional gel. Electrophoretic conditions in the second dimension were the same as those of Figs. 1 and 2. * RCAM-glycinin from cv Amsoy.

sion, which seems to correspond to cv Amsoy glycinin, gave the same extra major and minor subunit bands as those of cv Amsoy RCAM-glycinin on the two 2-D gels. The results demonstrate that the extra major subunit is a true subunit of cv Amsoy glycinin and indicate that the extra minor subunit may be a part of the glycinin molecule. A similar result has been obtained with the purified glycinin from cv Norin No. 1.

From the above results and discussion it can be concluded that there exist varietal differences of the subunit composition of glycinin and that it is certainly polymorphism of the protein. Polymorphism of storage proteins of leguminous seeds has been reported in *Arachis hypogaea* [9], *Pisum sativum* [10] and *Phaseolus vulgaris* [11]. In preliminary investigation, we have reported that there exist some varietal differences of the subunit composition of glycinin [12]. So far as we are aware, this is the first time that polymorphism of glycinin has been demonstrated in soybean seeds. Existence of the extra subunit of glycinin suggests that the protein molecule of the glycinin is heterogeneous.

EXPERIMENTAL

Soybeans (cvs Raiden, Tokyo, Norin No. 1 and Amsoy) were grown at the Iwate Univ. Agronomy Farm, Morioka in 1977. Purification of glycinin from Et_2O-defatted flakes was performed as described in ref. [5]. Each purified protein showed a single symmetrical sedimentation peak by ultracentrifugal analysis (not shown). Reduction and carboxyamidemethylation of the proteins were carried out as described in ref. [13]. Disc PAGE with urea was performed using discontinuous buffers diluted ×3 [6, 7]. SDS slab PAGE was performed using a discontinuous buffer system [14]. A separation gel with a gradient concn from 10 (top) to 17.5% (bottom) of acrylamide was prepared from a stock soln (30 g acrylamide, 0.8 g *N,N'*-methylenebisacrylamide in 100 ml H_2O). 2-D PAGE was performed using disc PAGE (2.5 × 75 mm) in the first dimension and slab PAGE with urea or the SDS slab PAGE in the second dimension. The gels were stained with 0.3% Coomassie Blue R-250 in H_2O–MeOH–HOAc (9:9:2) and destained with H_2O–MeOH–HOAc (7:2:1).

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