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Equal Expression of the Maternal and Paternal Alleles for the Polypeptide Subunits of the Major Storage Protein of the Bean *Phaseolus vulgaris* L.[1]

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TIMOTHY C. HALL, ROBERT C. MCLEESTER, AND FREDRICK A. BLISS
Department of Horticulture, University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT

Discontinuous sodium dodecyl sulfate slab gel electrophoresis of G1 globulin from several strains of *Phaseolus vulgaris* L. seed permitted clear resolution of the constituent polypeptides. Three strains (Tendergreen, Canadian Wonder, and BBL 240) had subunits of molecular weight 53,000, 47,000 and 43,000 while two strains (Seafarer and PI 229,815) had 50,500, 47,000 and 43,000 molecular weight subunits. F_1 seed from the cross BBL 240 × PI 229,815 showed four polypeptides on dissociation of the G1 protein; however, the amount of each of the 53,000 and 50,500 subunits was half that of the 47,000 subunit. This is interpreted as evidence that both the maternal and paternal loci for these polypeptides are transcribed and translated with similar efficiency. All of the polypeptides were found to have associated sugar residues.

We have shown previously that G1 globulin, the major storage protein of French bean (*Phaseolus vulgaris* L.) seeds, has three polypeptide subunits (4, 10). These subunits are synthesized by classical ribosomal systems, and each appears to be an individual gene product, there being no evidence for posttranslational cleavage of a large precursor molecule (9). One of the strains studied, PI 229,815[2] was found to have a modified subunit composition in which the largest subunit was nearly the same size as the middle subunit (5). Thus, after electrophoretic separation on continuous SDS acrylamide gels, the polypeptide pattern appeared two-banded, while that of the cultivar Tendergreen was clearly three-banded.

Using different conditions for electrophoresis we now show unequivocally the three-banded nature of the modified strain and provide a model for the phenotypic expression of the large subunit, which is inherited as expected for a polypeptide controlled by a single Mendelian gene (5). We also confirm that each of the polypeptide subunits has associated sugar residues.

MATERIALS AND METHODS

Plant Material. Bean (*P. vulgaris* L.) seeds of the cultivars BBL 240 and Seafarer and of PI 229,815 were obtained from stocks held in the breeding program of F. A. Bliss. Canadian Wonder was obtained from Thompson and Morgan, Ipswich, England and Tendergreen from the L. L. Olds Seed Co., Madison, Wis.

G1 Globulin Extraction. Dry mature seeds were covered with a small amount of glass wool and ground with a pestle and mortar to a flour using a freshly broken Pasteur pipette as a grinding aid. Protein was extracted at room temperature by addition of 0.5 M NaCl in 0.025 N HCl (20 ml/g flour). The suspension was centrifuged (J-20 rotor on a Beckman J-21 centrifuge) at 20,000 rpm for 15 min and the pellet was discarded. Five volumes of distilled H_2O (O C) were added to the clear supernatant. The precipitated globulin was sedimented at 20,000 rpm for 10 min and finally dissolved in 0.5 M NaCl. The protein concentration was determined by UV absorption, assuming $A_{278}^{0.1\%}$ protein = 1. Prior to electrophoresis in the G1 protein was dissociated by heating for 2 min at 100 C with an equal volume of cracking buffer (3), 100 ml of which contained: 10 ml 625 mM tris-HCl (pH 6.8), 2 g SDS, 2 ml β-mercaptoethanol, 40 g sucrose, 10 μg bromophenol blue, and 1 ml 0.2 M EDTA.

Discontinuous SDS Slab Gel Electrophoresis. Slab gels (0.75 mm thick) were prepared according to Laemmli (3) as modified by Knowland (2) to have an acrylamide to bisacrylamide ratio of 200:1, and run in an apparatus similar to that of Studier (7) using a 20-well comb (Hoefer Scientific Instruments Inc., San Francisco). The sample (3 μg protein in 3 μl) was added and a constant current of 10 mamp applied for 45 min, then increased to 25 mamp until the front was about 1 cm from the end of the gel. The anode was at the bottom of the gel. For detecting sugar residues 27 μg protein were applied.

After electrophoresis, the stacking gel was removed and the running gel washed off the glass plate into a fine mesh aquarium fish net (10 × 12.5 cm), then transferred to a 1-liter beaker containing 0.2% (w/v) Coomassie brilliant blue R in 9% (v/v) acetic acid and 45% (v/v) methyl alcohol. The gel was stirred using a 5 cm Teflon stir bar on a magnetic stirrer for 15 min. Longer staining times (up to 30 min) were required as the stain solution aged.

Destaining was accomplished by stirring (3 hr) in a 2-liter beaker containing 7.5% (v/v) acetic acid and 25% (v/v) methyl alcohol. The destaining solution was circulated through a charcoal filter cartridge (model D8904, Barnstead Co., Boston) previously saturated with acetic acid and methanol.

Gels increased in size after staining and destaining from 145 × 115 mm (wide × long) to 185 × 155 mm. Prior to drying, the gels were stirred (15 min) in destaining solution to which glycerine (3%, v/v) had been added, then placed on 3MM Whatman filter paper (presoaked in the destain-glycerine solution), and dried as described by Studier (7). The glycerine treatment leaves the dried gel flexible so that it does not crack on storage.

Periodic Acid-Schiff Staining for Sugar Residues. This was

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[2] Abbreviation: PI: plant introduction.

done according to Kapitany and Zebrowski (1), except that stirring the thin slab gels in the staining and destaining solutions (300 ml each) permitted considerable reduction in time. Thus, after electrophoresis, the gels were stirred in 12.5% (w/v) trichloroacetic acid for 30 min; in 1% (w/v) periodic acid for 1 hr; 15% (v/v) acetic acid for 1 hr (three changes); Schiff reagent (Fisher Scientific Co., Fair Lawn, N.J.) for 2 hr, and finally destained overnight in 15% (v/v) acetic acid (14 hr, three changes). The last two steps were in the dark at 4 C.

RESULTS AND DISCUSSION

Strong evidence that seeds having an apparently two-banded electrophoretic pattern for G1 protein contained three polypeptide subunits was presented previously (5). However, we were unable to resolve these subunits completely using our standard electrophoretic procedures. Using the discontinuous SDS gel system described under "Materials and Methods," we have obtained excellent separation of the polypeptide subunits of G1 globulin from seeds of several strains (Fig. 1).

G1 globulin from cv. Tendergreen was used as a reference sample in lanes A, E, I and M of Figure 1. The polypeptides of G1 globulin of BBL 240 (lane B) were identical in mobility to those of Tendergreen, as were those from the commercial variety Canadian Wonder (lane F). The mol wt of these peptides are 53,000, 47,000, and 43,000 (4). G1 protein from PI 229,815, which had previously appeared two-banded, was clearly three-banded (lane D), the largest polypeptide migrating faster than that of Tendergreen, BBL, 240 and Canadian Wonder. In this cultivar, the largest subunit was a lower mol wt, 50,500 (Fig. 2). The G1 protein from cv. Seafarer also yielded a 50,500 mol wt large subunit (lane G).

F_1 progeny from crosses between the parental lines carrying the 53,000 and 50,000 mol wt polypeptides will be heterozygous for this large subunit of G1 protein. If both maternal and paternal alleles are expressed, then both the 53,000 and 50,500 mol wt subunits should be present in F_1 seeds; this was confirmed for the cross PI 229,815 × BBL 240 (lanes C and K). The reciprocal cross BBL 240 × PI 229,815 yields an identical profile. The relative intensity of the stained polypeptides was consistent with the model presented in Figure 3 for the inheritance of G1 subunits. In the parental lines, the largest polypeptide (53,000 or 50,500 mol wt subunit) appeared as darkly stained as the 47,000 subunit, while the F_1 seed showed approximately half the intensity of stain for the 53,000 and 50,500 subunits compared with the 47,000 subunit. Thus, the F_1 seed of this cross contains four subunits, and the difficulty in resolving these polypeptides of very similar mol wt accounts for the smeared appearance of the "intermediate" phenotype reported previously (5). The relative amounts of each peptide suggest that the alleles derived from each parent are transcribed and translated with equal efficiency. Artificial mixtures (1:1) of BBL 240 + PI 229,815 produced a four-banded pattern (lane J) identical to that of the cross (lanes C and K), further supporting the model shown in Figure 3. A mixture (1:1) of G1 protein from Canadian Wonder + Seafarer also gave a four-banded pattern (lane L).

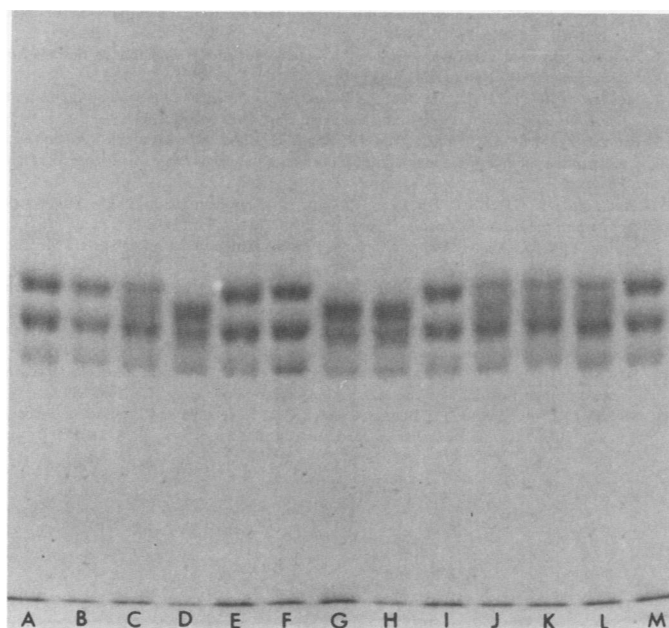

FIG. 1. Electrophoretic separation of G1 protein derived from several seed lines. Protein from seeds of the cv. Tendergreen was run in lanes A, E, I, and M; from BBL 240 in lane B; from F_1 seed of the cross PI 229,815 × BBL 240 in lanes C and K; from PI 229,815 in lanes D and H; from cv. Canadian Wonder in lane F; and from cv. Seafarer in lane G. Mixtures of equal volumes of extracts from BBL 240 and PI 229,815 were run in lane J, and of Canadian Wonder and Seafarer in Lane L. The top of the gel is not shown; some protein ran with the front and is seen at the bottom of the photograph (+ electrode).

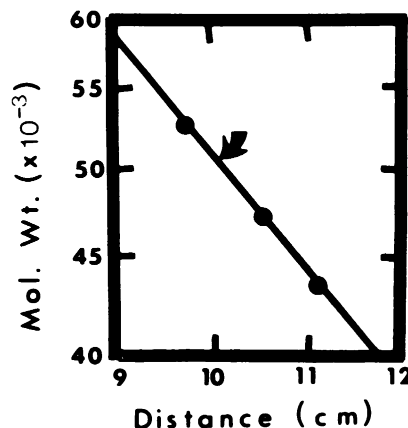

FIG. 2. Determination of the mol wt of the shortened version of the large subunit. The distance migrated by the three subunits (dots) of the cv. Tendergreen was plotted against a log scale of their known mol wt. The arrow marks the distance migrated by the large subunit of G1 from PI 229,815. Data were taken from lanes I and K of Figure 1.

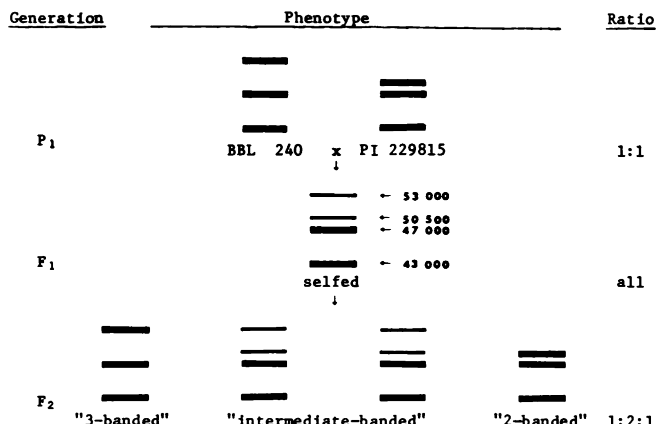

FIG. 3. Model explaining the electrophoretic appearance of G1 protein in progeny of the cross BBL 240 × PI 229,815, which shows classical Mendelian co-dominance. The thickness of the bands indicates gene expression: — expression of a single allele: — expression of two alleles (one from each parent). The terms "2-banded", "intermediate-banded," and "3-banded" refer to the appearance of these phenotypes in a previous study (5). Mol wt of the polypeptides are shown for the F_1 generation.

While we expect the G1 monomer to be comprised of a 1:1:1 ratio of each of the subunits, it is apparent from numerous extracts that the fastest migrating subunit (43,000 mol wt) always stains less intensely than do the others (see Fig. 1). Ultraviolet scans (280 nm) of unstained gels also indicate lower amounts of the small subunit than of the other subunits. This apparent disparity could result from a low content of aromatic amino acids in the small polypeptide and resultant different staining characteristics. The quantitative recovery of G1 subunits by an affinity technique (6) which depends on the reversible monomer-tetramer association of G1 globulin (11) would seem to support a 1:1:1 ratio for the subunits, but this point is yet to be unequivocally established.

G1 globulin is known to be a glycoprotein (8) and periodic acid-Schiff staining of the subunits after electrophoresis revealed that each polypeptide had associated sugar residues (Fig. 4). The lack of sharpness of the bands results from the rather small amount of sugars bound to the polypeptides (about 3% by weight), the relatively insensitive staining technique, and the consequent need for application of rather large amounts of sample proteins. The relative amount of stain for the smallest subunit was less than that for the other subunits, indicating either lower levels of associated sugar, or the presence of smaller amounts of this subunit. While different sugar moieties do not account for the differences in mobilities of the 53,000 and 50,500 subunits, we have observed that the polypeptides from some preparations appear as doublets. It is possible that these doublets result from varying levels of sugar residues attached to the polypeptides. The distance between the bands of these doublets would indicate a maximum difference in mol wt of 1,500. We have not excluded the possibility that the doublets arise from some property of the electrophoretic system. It is also conceivable that multiple closely linked genes for the polypeptides could give rise to this situation.

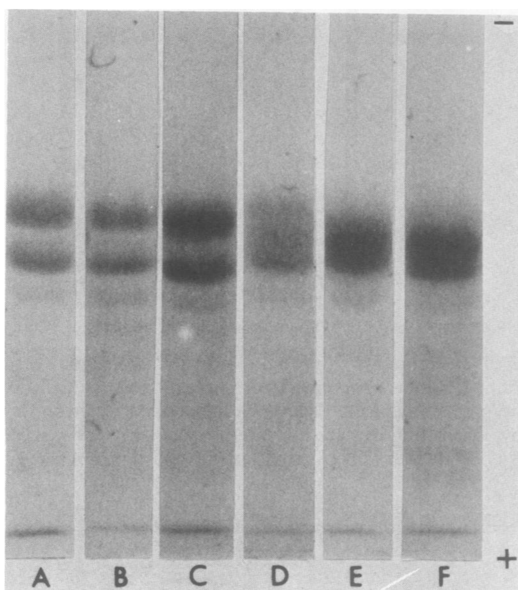

FIG. 4. Sugar residues of the G1 polypeptides. Electrophoretically separated G1 protein from cv. Tendergreen (lane A), BBL 240 (lane B), cv. Canadian Wonder (lane C), F_1 seed of the cross PI 229,815 × BBL 240 (lane D), cv. Seafarer (lane E), and PI 229,815 (lane F) was stained by the periodic acid-Schiff procedure.

CONCLUSIONS

We have now established that the major storage protein of *P. vulgaris* consists of three polypeptide subunits which reversibly associate according to pH conditions between polypeptide, monomer, and tetramer configurations. Each polypeptide has associated sugar residues. The subunits of Tendergreen, Canadian Wonder, and BBL 240 mol wt of 53,000, 47,000, and 43,000; those of PI 229,815 and cv. Seafarer are 50,500, 47,000, and 43,000. Progeny of crosses between these two groups express both forms of the large polypeptide, the amount of each being half that present in lines homozygous for one or the other form.

The nature of the molecular event (deletion or addition of nucleotides in the messenger RNA, differing initiation or termination sites, etc.) resulting in the change of size for the large subunit has yet to be ascertained. Possibly the larger version of this subunit contains a methionine residue; this would account for the generally higher methionine content we have observed for seeds having the 53,000 rather than the 50,500 mol wt subunit.

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