Heritable Variation in a Polypeptide Subunit of the Major Storage Protein of the Bean, *Phaseolus vulgaris* L.  

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ABSTRACT

Electrophoretic analysis of the major seed protein, G1 globulin, from four strains of *Phaseolus vulgaris* L. revealed a three-banded pattern for two strains having a high methionine content (BBL 240 and PI 302,542). The other two strains (PI 207,227 and PI 229,815) known to have a lower seed methionine content, had a two-banded subunit pattern for the G1 globulin. Analytical ultracentrifugation confirmed that globulin from the two-banded strains underwent pH-dependent reversible dissociation similar to that previously found for a three-banded cultivar; additionally, the protomer molecular weight showed that three subunits of about 50,000 molecular weight each were present in the G1 globulin of the two-banded strain. Gel patterns of G1 globulin from the two strains used as parents, BBL 240 and PI 229,815, showed differences in the largest subunit, which appeared as either a 53,000 molecular weight polypeptide known to be present in the three-banded strain, or as a shorter polypeptide having a molecular weight close to 47,000. Analysis of G1 protein from portions of single hybrid seeds showed a banding pattern intermediate between the two- and three-banded types. The subunit pattern from all seeds with intermediate-banded parents segregated in a manner consistent with that expected for control of the polypeptide by a single Mendelian gene. The remaining portions of the seeds were grown to confirm that they represented true crosses. The procedures used are essentially nondestructive, and can be used as a basis for selecting seeds having different protein characters.

While soybeans have the highest protein content and economic value, the oil present in these seeds is a hindrance to protein studies, and seeds of *Phaseolus vulgaris* L. were selected as the preferred material.

Dry seeds of *P. vulgaris* contain 20 to 30% protein by weight, depending on the cultivar (5). Of this protein, 50 to 75% is globulin (requiring appreciable salt for solubility) which can be separated into two fractions. In Osborne's studies on legumes he adopted the term legumin (first used by Braconnot in 1827) for the fraction requiring the highest level of salt for solubility, and coined the term vicillin for the fraction soluble in lower salt concentrations (9). Through the years, misuse of these terms has occurred; they have been applied to proteins from many genera despite the differing properties of protein in a given fraction from species to species. To avoid the resulting confusion, we designated the globulin fraction which first precipitates on addition of water to a saline solution of *P. vulgaris* protein as the G1 fraction, and the second fraction (obtained by extensive dialysis) as the G2 fraction (7).

Despite the apparently simple separation of the two globulin fractions on the basis of solubility, great difficulty has been experienced in obtaining them free from traces of cross-contamination. We developed an acidic extraction procedure (7) which achieves this goal, permitting characterization of the G1 and G2 globulin fractions (15). Recent articles (12, 18) have described separation of globulins using zone precipitation, which performs the same function as our isoelectric separation, but is lengthier and limits the amount of material that can be handled.

Although the G2 fraction gives a single boundary sedimenting at 6.6S in the analytical ultracentrifuge (16), it is not homogeneous, and contains several growth-inhibiting substances. This fraction has a high content of sugar-containing proteins, and a major component, glycoprotein I (10), possesses hemagglutinating properties, i.e., it is a lectin. Purification of lectin from *Phaseolus* that is lethal to quail has also been described by Andrews and Jayne-Williams (1).

The G1 fraction is a homogeneous nonhemagglutinating globulin identical to that described as glycoprotein II by Pusztai and Watt (11), with the major sugar components being D-mannose and D-glucosamine. Depending upon pH conditions G1 globulin reversibly dissociates between tetrameric (18.2S at pH 3.8), protomeric (7.1S at pH 7), and polypeptide (3.0S at pH 12) configurations (16). Unbuffered NaCl extracts have an acidic pH, suggesting that the protein is stored predominantly in the tetrameric state. At neutral pH the protomeric form predominates and it is readily confused with G2 protein when subjected to analytical ultracentrifugation (see 16). This accounts for previous reports that 7S globulin is the major storage protein of *P. vulgaris* seeds; in fact G1 is the most abundant protein, accounting for 76% of the total globulin component in the cultivar Tendergreen (15).

G1 protein from Tendergreen seeds contains three different
polypeptide subunits, having mol wt of 53,000, 47,000, and 43,000 (7). We now describe variation in the subunit composition of G1 globulin from different strains of *P. vulgaris*, and show that for two of these strains the largest subunit segregates as expected for a peptide controlled by a single Mendelian gene.

**MATERIAL AND METHODS**

**Plant Material.** Selection of the four *Phaseolus vulgaris* strains used in this study was based on heritable differences of seed protein and methionine content (5, 6). BBL 240 and PI 302,542 were strains high in methionine (1.2 and 1 g methionine/100 g protein), while PI 207,227 and PI 229,815 were lower in methionine (0.92 and 0.86 g methionine/100 g protein). Previous characterizations of the major seed storage protein, G1 globulin, used the cultivar Tendergreen (3, 7, 13, 15, 16). Tendergreen was, therefore, included as reference material in the present work.

**Seed Preparation.** Testas were removed from mature, dry seeds and the cotyledons carefully separated. One half of each seed, which consisted of the embryonic axis attached to one cotyledon, was germinated in Perlite and the resulting plant produced seeds of the next generation. The other cotyledon was used for extraction of seed protein. This procedure resulted in nearly 50% mortality. However, removal of a portion of the seed by cutting across the seed distal to the embryo and above the lens (Fig. 1) resulted in nearly 100% survival. It was important to germinate the sectioned seed on porous material, to maintain the temperature during germination close to 25 C (not above 26 C), and to water sparingly until emergence. Each seed of the parental lines and the progeny resulting from various hybridizations was handled in the above manner. Protein characterization is reported for individual half-seeds or seed-sections. The seed-section method was used for data on F1 seeds, and the half-seed procedure for all other experiments.

**Protein Extraction.** Each of the half-seeds or seed-sections was weighed and ground in a mortar. The flour was washed into a centrifuge tube with a measured volume of 0.5 m NaCl containing 0.25 m ascorbic acid, pH 2.4. Two milliliters of solution were used for each 100 mg of seed flour. The protein was extracted with intermittent stirring for 3 hr at 25 to 27 C, then centrifuged at 20,000 rpm in a JA-20 rotor on a Beckman J-21 centrifuge for 15 min. The supernatant was decanted immediately into five volumes of distilled H2O (4 C) when a white precipitate of the G1 globulin was obtained. This was pelleted by centrifugation (20,000 rpm for 15 min at 4 C), then redissolved in 0.5 m NaCl and washed free of ascorbate by a further cycle of centrifugation. The G1 pellet was finally dissolved in 0.5 m NaCl, the volume being one-fifth that of the original extraction solution. Protein purity and concentration (A280/1 m = 10) were determined from the UV absorption spectrum on a Beckman DK 2A spectrophotometer. All purified G1 samples were adjusted with 0.5 m NaCl to a protein concentration of 5 mg/ml.

**Electrophoresis.** The G1 protein was dissociated into its polypeptide subunits by adding an equal volume of a solution containing 9 m urea, 3C (w/v) SDS, and 3C (v/v) 2-mercaptoethanol and heating for 3 min in a boiling water bath. The subunits were electrophoretically separated on 5C (w/v) SDS-urea acrylamide gels as described previously (14). Gels were run at 9.5 mamp, tube for 6.5 hr. The separated peptides were stained with Coomassie brilliant blue (17).

**Analytical Ultracentrifugation.** This was done as described previously (16).

**RESULTS**

**Polypeptide Profiles of G1 from Different Strains.** Representative electrophoretic profiles of polypeptides of G1 protein from Tendergreen and the four strains are shown in gels a to e of

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**Fig. 1.** Diagram of seed sections and half-seeds used for analysis. On the left the visible anatomical features are shown together with the position (marked cut) at which the seed was sectioned. The upper section was used for protein analyses, and the lower section for regeneration. On the right a half-seed used for regeneration and containing the cotyledon and embryonic axis is shown. The other half-seed was used for protein analysis.

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**Fig. 2.** Polypeptide subunit of G1 protein from several strains of *Phaseolus* separated on SDS acrylamide gels. Patterns are shown for (a) Tendergreen (three-banded); (b) PI 207,227 (two-banded); (c) PI 302,542 (three-banded); (d) BBL 240 (three-banded); (e) PI 229,815 (two-banded); (f) F1 (intermediate-banded) from the cross BBL 240 X PI 229,815; (g) F1 (intermediate-banded) from the cross PI 229,815 X BBL 240. Migration was from top (cathode) to bottom (anode).
Figure 2. The profiles of PI 207,227 and PI 229,815 appeared to have only two subunits, whereas those of PI 302,542 and BBL 240 had three-banded profiles similar to that of Tendergreen. These characteristic banding patterns were invariant over locations and years as determined by tests on 13 half-seeds of each line from several sources.

Evidence That Apparently Two-banded Seeds Contain Three Subunits. The possibility that one of the polypeptide subunits of G1 globulin could readily be lost without disturbing the reversible dissociation character (16) seemed remote. An alternative explanation was that the largest subunit (mol wt 53,000) was modified so that it co-electrophoresed with the middle subunit (mol wt 47,000). Strong evidence that this was the case was obtained by analytical ultracentrifugation of the G1 protein from PI 229,815. The mol wt of the protomeric form was 159,000 ± 24,000, which is consistent with its having three subunits, each of approximately 50,000 mol wt. Further, the G1 protein showed reversible dissociation between peptide, protomeric, and tetrameric forms (Fig. 3) similar to that found previously for the cultivar Tendergreen (16), which also shows a three subunit pattern (gel a, Fig. 2).

Genetical Evidence for Inheritance of Modified Subunit. The clear distinction between the two- and three-banded characters made genetic analysis essential. Therefore, the hybrid BBL 240 × PI 229,815 and the reciprocal hybrid were made (Table 1). All F1 seeds showed banding patterns for G1 protein which were intermediate (gels f and g, Fig. 2) between the two- and three-banded phenotypes of the parental strains. All F1 seeds analyzed were confirmed to be true hybrids. The test was the phenotypic expression of hypocotyl color of plants grown from the seed sections containing the embryonic axis. BBL 240 is without purple pigment, whereas PI 229,815 has deep purple leaf veins and stem. Hybrids of crosses between these lines produce pale purple stems and veins. This test is an especially valuable control since P. vulgaris is almost exclusively self-pollinated.

The F1 plants were allowed to self-pollinate and G1 protein

![Diagram](image-url)

**Fig. 3.** Analytical ultracentrifugation of G1 protein from PI 229,815 showing pH-dependent reversible dissociation of an apparently two-banded strain. The saline-washed tetramer protein is shown in (a), and dissociation to protomer (b) and peptide (c) forms are shown as the pH of the sample was increased by addition of NaOH. Decreasing the pH by addition of HCl caused association to the protomer (d) and tetramer (e) forms. Photographs were taken at 16-min intervals after reaching 64,550 rpm and are printed so that the direction of sedimentation is from left to right. The meniscus of the sample cell has been aligned so that the boundary for each sedimenting species is vertically aligned. The $s_{20, w}$ values are indicated for exposures taken at 32 min.
from sections of 55 seeds of the resulting F2 generation was electrophoretically analyzed for subunit composition. The observed segregation ratio among F2 seeds based on the gel patterns did not deviate statistically from a predicted 1:2:1 (three-intermediate-two-banded) ratio (Table I). This suggests that variability in the large subunit of G1 protein is under the control of a single Mendelian gene. This hypothesis was tested and confirmed by analyzing the seeds of the F3 progeny obtained by selfing the F2 plants (Table I). Thirty of the F2 seeds were analyzed by the half-seed method, but only 15 survived to become parents, which accounts for the small number of F3 families. A meaningful chi-square test cannot be performed on the segregation ratio for a population of this size due to the sampling error involved. The number of progeny (85 seeds) from the 11 F3 families having an intermediate-banded parent was ample for a valid chi-square evaluation of the segregation observed. No segregation occurred in the F3 families from the F2 parents having the distinct two- or three-banded character.

**DISCUSSION**

The genetic control of a single subunit of G1 globulin revealed in this study by analysis of the electrophoretic banding pattern provides a valuable basis for future plant breeding studies. Seed of the parental bean strains used was known to differ in protein content and amino acid composition (5, 6). The electrophoretic profile for the two strains high in methionine (BBL 240 and PI 302,542) was typically three-banded, whereas that for the lower-methionine strains (PI 229,815 and PI 207,227) was two-banded (Fig. 2). If a useful correlation between the appearance of G1 globulin subunits and seed methionine content exists, electrophoretic screening for this character will greatly assist in the improvement of legume protein. Preliminary experiments (J. Romero, unpublished) show that it is not necessary to purify the G1 globulin (as was done in this study) for large scale screening, since differences in the polypeptide banding pattern can be seen from crude extracts of the total seed. As shown (Fig. 1), sufficient material can be obtained for electrophoretic analysis without destroying the seed; therefore, seed having enhanced characters can be grown to maturity.

Previous studies on pea seed by Davies (2) indicated that maternal and paternal factors can influence the protein composition in progeny seed. Maternal effects can include growth conditions, cytoplasmic genetic factors, and nuclear gene products present in the cytoplasm. While we have some preliminary data suggesting that these effects can occur in Phaseolus, the data shown in Table I unequivocally exclude such possibilities for hybrids between BBL 240 and PI 229,815. If maternal influences were operating, the banding pattern of G1 protein from the F1 seed would resemble that of the female parent. This was not the case since all F1 seeds had an intermediate-banding pattern (gels I and g, Fig. 2, and Table I).

At the molecular level, the simplest explanation for the observed banding patterns is that PI 229,815 is homozygous for a gene which transcribes and translates to yield a polypeptide subunit smaller by about 50 amino acid residues than the 53,000 mol wt subunit coded by the allele in BBL 240. The F1 seed is heterozygous at this locus, and it appears that each allele is translated in roughly equal amounts. In an accompanying article the cell-free translation of the subunits of G1 protein is reported (13). As mentioned above, there are indications that some strains of P. vulgaris show maternal and other influences on the inheritance of the subunits of the major seed protein. Confirmation of the mechanisms controlling these differences in G1 protein subunits should be possible by refinement of the studies in vitro. Combination of biochemical evidence with genetical data will provide a sound basis for meaningful improvement of the nutritional value of legume protein.

**LITERATURE CITED**


