Theor Appl Genet (1985) 71:351–358

Inheritance and biochemical analysis of four electrophoretic variants of β-conglycinin from soybean *

C. S. Davies, J. B. Coates and N. C. Nielsen

United States of Department of Agriculture, Agricultural Research Service, Agronomy Department, Purdue University, West Lafayette, IN 47907, USA

Accepted May 13, 1985
Communicated by D. von Wettstein

Summary. Three genes which code for variant β-conglycinin subunits were identified. Alleles Cgy_1^S and Cgy_2^S were codominant with Cgy_1 and Cgy_2 and produced α′ and α subunits, respectively, with reduced electrophoretic mobility. Allele Cgy_3^D increased the mobility of at least one polypeptide in the β subunit family and exhibited incomplete dominance. Gene loci Cgy_2/Cgy_2^S and Cgy_3^D/cgy_3^D were linked, whereas $Cgy_1/Cgy_1^S/cgy_1$ segregated independently of the others. Techniques developed for purification of normal β-conglycinin subunits were effective in purifying the altered subunits. Deglycosylated variant proteins from seeds containing the alleles Cgy_1^S, Cgy_2^S, or Cgy_3^D also has altered mobility relative to deglycosylated normal proteins. Therefore, the altered subunits contained changes in their amino acid sequences rather than in their carbohydrate moieties. This interpretation is consistent with the observed codominant or incompletely dominant mode of inheritance for these alleles and suggests that each contains an altered nucleotide sequence in the structural gene. A fourth variant, which exhibited doublet α′ and α electrophoretic bands, was inherited in a recessive fashion. Deglycosylated subunit proteins from this variant were identical in electrophoretic mobility to those of the deglycosylated normal protein. This suggests that the doublet phenotype resulted from an alteration in the carbohydrate moiety of these subunits. The gene or genes which condition this variant presumably are required for normal post-translational modification of the subunit carbohydrates and as such may be useful for investigating these events.

* Cooperative research of USDA-ARS and the Indiana Agric. Exp. Stn., Purdue Univ., West Lafayette, IN 47907, USA. Indiana Agric. Exp. Stn. Journal Article 10,323. Financial support from the American Soybean Research Foundation is gratefully acknowledged

Key words: β-Conglycinin mutant – Soybean – Codominance – Incomplete dominance – Linkage

Introduction

β-Conglycinin (7S protein) constitutes about 30% of the total protein of soybean seeds. It is isolated from seed extracts as a trimer (Thanh and Shibasaki 1978) composed of various combinations of at least four subunits, denoted α′, α, β and β′ (Coates et al. 1985). Each subunit is glycosylated and undergoes a complicated series of co- and post-translational modifications during synthesis (Sengupta et al. 1981; Barton et al. 1982).

A null-allele, cgy_1, for the α′ subunit of β-conglycinin (Kitamura et al. 1984) segregates independently of gy_4, a null-allele for the $A_5A_4B_3$ subunit of glycinin, the other major storage protein of soybean seeds. We now report the identification, inheritance, and linkage relations between three genes that confer aberrant electrophoretic mobility of the α′, α, and β subunits of 7S protein. A fourth variant is described which confers doublet α and α′ subunits. The variant subunits were each purified and deglycosylated with trifluoromethanesulfonic acid (TFMS) in order to investigate the structural basis of the mutations.

Materials and methods

1 Genetic studies

Four Plant Introductions whose 7S proteins exhibited unusual electrophoretic banding patterns (P.I. 54.608-1, "α^{s}"; P.I. 90.567-1, "$\alpha_2\alpha_2'$"; and P.I. 81.041-1 or P.I. 88.302-1, "β_2") were identified by screening part of the USDA Northern Germ-

plasm Collection (maintained by R. L. Bernard, USDA/ARS, Agronomy Dept., Univ. Illinois, Urbana, IL 61801, USA). Each line was propagated for several generations in both the greenhouse and the field so that the seeds could be checked periodically for consistency of phenotype. The inheritance of the "β_2" and "α^S" phenotypes was investigated by analysis of electrophoretic patterns in the F_1, F_2, and F_3 progeny of crosses between Century (a locally-adapted cultivar with an electrophoretic pattern considered normal) and P.I. 81.041-1 and P.I. 54.608-1, respectively. Information on the inheritance of the "$\alpha_2\alpha_2'$" phenotype was obtained by analysis of F_1 seeds from the crosses P.I. 90.567-1 × P.I. 54.608-1 and P.I. 90.567-1 × Century. The electrophoretic patterns of seed extracts prepared from the F_2 progeny of the crosses P.I. 54.608-1 × P.I. 88.302-1 and P.I. 88.302-1 × P.I. 81.041-1 were analyzed in order to investigate linkage relations and possible interactions between genes controlling the aberrant bands.

Seeds were nondestructively sampled by slicing them with a razor blade to remove 15–20 mg of the cotyledon. Sample preparation and electrophoretic analysis were initially by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Kitamura et al. (1984). It was later found that the addition of urea to SDS-polyacrylamide gels as described by Fontes et al. (1984) permitted resolution of an additional variant pattern, "α'^S", in P.I. 54.608-1. Genetic data for this variant were then obtained by analysis of remnant seed from the F_2 and F_3 generations of the above crosses between P.I. 54.608-1 and Century. Additionally, the genetic relationship between the "α'^S" phenotype and cgy_1, a null-allele of the structural gene for the α' subunit (Kitamura et al. 1984), was investigated by analysis of F_2 progeny from the cross P.I. 54.608-1 × 'Keburi'.

2 Biochemical studies

7S proteins from CX 635-1-1-1 (a breeding line with typical 7S gel pattern), P.I. 54.608-1, P.I. 90.567-1, and P.I. 81.041-1 were purified by isoelectric precipitation (Thanh and Shibasaki 1976), filtration on Sepharose 6B, and specific elution of the 7S glycoproteins from ConA-Sepharose 4B with 0.1 M α-methyl-D-mannoside (Coates et al. 1985). The proteins were deglycosylated by a method adapted from Edge et al. (1981) as follows. Lyophilized protein (10 mg) was suspended in 0.3 ml anisole in a screw-capped Corex tube, and the mixture was cooled to 0 °C. Precooled (0 °C) trifluoromethanesulphonic acid (0.6 ml) was added and the suspension vortexed until the protein dissolved. This mixture was incubated with stirring at 0 °C for 2 h. The reaction was stopped by the addition of 15 ml precooled (–70 °C) diethyl ether containing 10% (v/v) n-hexane, and the mixture was kept at –70 °C for at least 1 h to allow the protein to precipitate. The protein was pelleted by centrifugation (3,000 rpm, 2 min) and then washed three more times with the diethyl ether-hexane solution. The protein was desalted by extensive dialysis against water and then lyophilized. Deglycosylated proteins were analyzed by SDS-PAGE as described earlier.

Results

1 Genetic studies

Inheritance of the "β_2" phenotype. The "β_2" phenotype (Fig. 1 A, lane 3) exhibited an extra band (β_2) directly

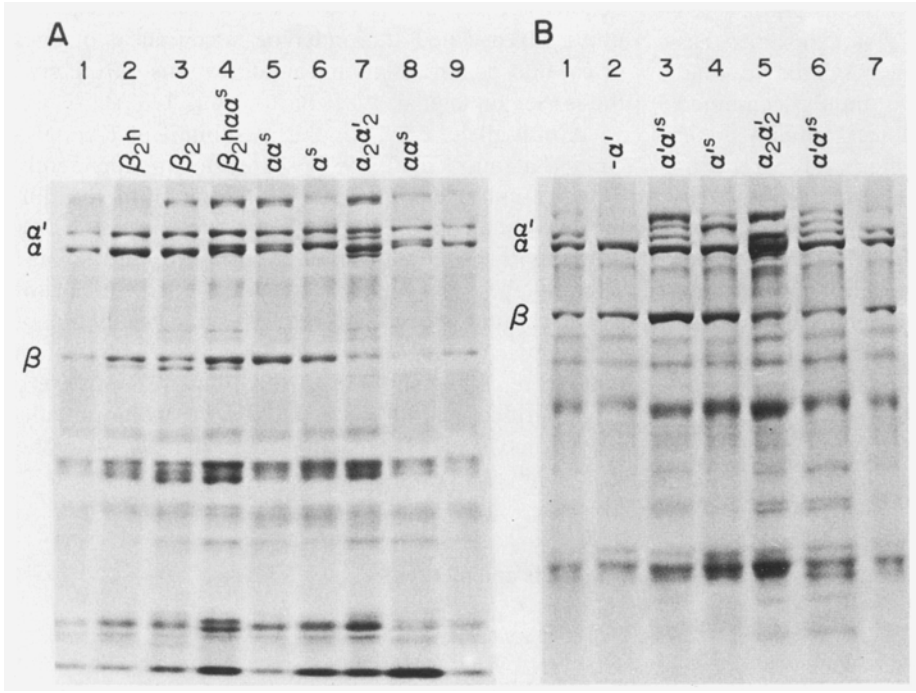

Fig. 1 A, B. Electrophoretic variants and their F_1 progeny. $\beta_2h = Cgy_3^D cgy_3^D$; $\beta_2 = Cgy_3^D Cgy_3^D$; $\beta_2h\alpha\alpha^S = Cgy_3^D cgy_3^S Cgy_2 Cgy_2$; $\alpha\alpha^S = Cgy_2 Cgy_2^S$; $\alpha^S = Cgy_2^S Cgy_2^S$; $\alpha_2\alpha_2' =$ P.I. 90.567-1; $-\alpha' = cgy_1 cgy_1$; $\alpha'\alpha'^S = Cgy_1 Cgy_1^S$; $\alpha'^S = Cgy_1^S Cgy_1^S$; $\alpha'\alpha'^S = Cgy_1 Cgy_1^S$. **A** SDS-polyacrylamide gel (7–13%); total seed protein: *1* Century; normal phenotype; *2* F_1 hybrid; normal "β_2" (Century × P.I. 81.041-1); *3* P.I. 81.041-1; "β_2" phenotype; *4* F_1 hybrid; "α^S" × "β_2" (P.I. 54.608-1 × P.I. 81.041-1); *5* F_1 hybrid; normal × "α^S" (Century × P.I. 54.608-1); *6* P.I. 54.608-1; "α^S" phenotype; *7* P.I. 90.567-1; "$\alpha_2\alpha_2'$" phenotype; *8* F_1 hybrid; "$\alpha_2\alpha_2'$" × "α^S" (P.I. 90.567-1 × P.I. 54.608-1); *9* Century; normal phenotype; **B** SDS-polyacrylamide gel (7–13%) + urea; total seed protein: *1* Century; normal phenotype; *2* Keburi; "$-\alpha'$" phenotype; *3* F_1 hybrid; normal × "α'^S" (Century × P.I. 54.608-1); *4* P.I. 54.608-1; "α'^S" phenotype; *5* P.I. 90.567-1; "$\alpha_2\alpha_2'$" phenotype; *6* F_1 hybrid; "$\alpha_2\alpha_2'$" × "α'^S" (P.I. 90.567-1 × P.I. 54.608-1); *7* Century; normal phenotype

Table 1. Observed and expected F_2 segregation ratios for the "β_2", "α^s" and "α'^s" phenotypes occurring in families from five different crosses

Cross	Parental phenotype	Genetic ratio	Observed and (expected) progeny ratio	P
1. Century × P.I. 81.041-1	"β" × "β_2"	1 : 3 ($\beta : \beta_2$h + β_2)	42 : 120 (40.5) (121.5)	> 0.88
		1 : 2 : 1 ($\beta : \beta_2$h : β_2)	42 : 71 : 49 (40.5) (81) (40.5)	> 0.20
2. P.I. 88.302-1 × P.I. 81.041-1	"β_2" × "β_2"	All β_2	80 (80)	–
3. Century [a] × P.I. 54.608-1	"$\alpha\alpha'$" × "$\alpha^s\alpha'^s$"	1 : 2 : 1 ($\alpha : \alpha\alpha^s : \alpha^s$)	55 : 130 : 58 (60.75) (121.5) (60.75)	> 0.54
		1 : 2 : 1 ($\alpha' : \alpha'\alpha'^s : \alpha'^s$)	66 : 172 : 82 (80) (160) (80)	> 0.19
		1 : 2 : 1 : 2 : 4 : 2 : 1 : 2 : 1 ($\alpha\alpha' : \alpha\alpha'\alpha'^s : \alpha\alpha'^s$: $\alpha\alpha^s\alpha' : \alpha\alpha^s\alpha'\alpha'^s : \alpha\alpha^s\alpha'^s$: $\alpha^s\alpha' : \alpha^s\alpha'\alpha'^s : \alpha^s\alpha'^s$)	9 : 29 : 7 (11.62) (23.25) (11.62) 19 : 57 : 27 (23.25) (46.5) (23.25) 10 : 23 : 5 (11.62) (23.25) (11.62)	> 0.16
4. P.I. 54.608-1 × Keburi	"α'^s" × α'	3 : 1 ($\alpha'^s : \alpha'$)	59 : 16 (56.2) (18.75)	> 0.56
5. P.I. 54.608-1 × P.I. 88.302-1	"$\alpha^s\alpha'^s$" × "β_2"	1 : 2 : 1 ($\alpha : \alpha\alpha^s : \alpha^s$)	19 : 45 : 18 (20.5) (41) (20.5)	> 0.67
		1 : 2 : 1 ($\alpha' : \alpha'\alpha'^s : \alpha'^s$)	23 : 40 : 19 (20.5) (41) (20.5)	> 0.80
		1 : 2 : 1 ($\beta_2 : \beta_2$h : β)	18 : 47 : 17 (20.5) (41) (20.5)	> 0.42
		1 : 2 : 1 : 2 : 4 : 2 : 1 : 2 : 1 ($\alpha\beta_2 : \alpha\beta_2$h : $\alpha\beta$: $\alpha\alpha^s\beta_2 : \alpha\alpha^s\beta_2$h : $\alpha\alpha^s\beta$: $\alpha^s\beta_2 : \alpha^s\beta_2$h : $\alpha^s\beta$)	18 : 1 : 0 (5.13) (10.25) (5.13) 0 : 45 : 0 : (10.25) (20.5) (10.25) 0 : 1 : 17 (5.13) (10.25) (5.13)	< 0.005
		1 : 2 : 1 : 2 : 4 : 2 : 1 : 2 : 1 ($\alpha'\beta_2 : \alpha'\beta_2$h : $\alpha'\beta$: $\alpha'\alpha'^s\beta_2 : \alpha'\alpha'^s\beta_2$h : $\alpha'\alpha'^s\beta$: $\alpha'^s\beta_2 : \alpha'^s\beta_2$h : $\alpha'^s\beta$)	6 : 13 : 4 : (5.13) (10.25) (5.13) 9 : 22 : 9 : (10.25) (20.5) (10.25) 3 : 12 : 4 (5.13) (10.25) (5.13)	> 0.92
		1 : 2 : 1 : 2 : 4 : 2 : 1 : 2 : 1 ($\alpha\alpha' : \alpha\alpha'\alpha'^s : \alpha\alpha'^s$: $\alpha\alpha^s\alpha' : \alpha\alpha^s\alpha'\alpha'^s : \alpha\alpha^s\alpha'^s$: $\alpha^s\alpha' : \alpha^s\alpha'\alpha'^s : \alpha^s\alpha'^s$)	7 : 9 : 3 : (5.13) (10.25) (5.13) 12 : 21 : 12 : (10.25) (20.5) (10.25) 4 : 10 : 4 (5.13) (10.25) (5.13)	> 0.95

[a] Includes reciprocal

beneath the band which corresponds to the β subunit on SDS-polyacrylamide gels. Our seed samples of P.I. 81.041-1 and P.I. 88.302-1 exhibited identical "β_2" gel patterns, and in both cases, the staining intensity of the β_2 band was similar to that of the normal β subunit band. This property was not noticeably altered after several generations of selfing or after production of seeds in either greenhouse or field environments.

Extracts of F_1 seeds from the cross Century × P.I. 81.041-1 also exhibited the β_2 band. However, unlike the parental "β_2" phenotype, the staining intensity of this band was lighter than that of the normal β subunit band (Fig. 1 A, lane 2). Banding patterns in the F_2 generation segregated approximately 1 : 2 : 1 ("β_2" : "β_2h" : "β"), where "β_2h" designates a phenotype which exhibits the lighter staining β_2 band, presumably

present in heterozygotes (Table 1, cross 1). Ten F_3 seeds from each of 39 selfed F_2 plants of known phenotype were then analyzed to test the hypothesis that an incompletely dominant allele of a single Mendelian gene controls the expression of this trait (Table 2, cross 1). As expected, all nine of the F_2 seeds with the "β" phenotype were true-breeding, and segregation for the β_2 and β bands was evident among progeny of all 17 seeds which were scored "β_2h". However, four of the 13 plants which were scored "β_2" (homozygous) had progeny which unexpectedly lacked a β_2 band. This was probably due to a misclassification of four "β_2h" (heterozygotes) into the "β_2" (homozygous) category, because the staining intensity relationship between the β_2 and β bands was occasionally difficult to score. This misclassification could also account for the slightly higher than expected number of "β_2" phenotypes (homozygotes) in the F_3 generation from this cross (observed: 59/174; expected: 43.5/174).

When seeds of the "β_2" and "β_2h" phenotypes were classified together, a good fit to a 3 : 1 ratio for presence *versus* absence of the β_2 band was obtained for the segregating F_3 seeds from the heterozygous F_2 plants (Table 2, cross 1) and for the F_2 progeny (Table 1, cross 1). These data supported the hypothesis that an allele of a single Mendelian gene (hereafter designated Cgy_3^D) caused the "β_2" phenotype. The specific mode of inheritance for this allele was, however, unclear. Results from two-dimensional electrophoresis of purified 7S proteins from CX635-1-1-1 indicated that β subunits extracted from normal seeds can be resolved into at least four spots (Nielsen, unpublished). Cgy_3^D could, therefore, be codominant to an allele "Cgy_3" which conditions a normal subunit. However, seed extracts from the β_2 phenotype, P.I. 81.041-1, and P.I. 88.320-1, contained one additional spot, which presumably corresponds to the aberrant protein observed in single dimension gels. Because none of the four 'normal' spots was missing in 2-D gel patterns of the β_2 phenotype, Cgy_3^D is probably inherited in a dominant rather than codominant fashion. Until the basis of the four spots from the β subunits in normal phenotypes can be resolved, we suggest that the gene symbols Cgy_3^D/cgy_3^D be used to describe the inheritance of this phenotype.

With this scheme, Cgy_3^D was completely dominant to cgy_3^D when classification was based solely on the presence or absence of the β_2 band. Additionally, the $Cgy_3^D\,Cgy_3^D$ and $Cgy_3^D\,cgy_3^D$ genotypes were correctly classified on the basis of staining intensity of the β_2 band relative to the β band in 26 out of 30 F_2 seeds that exhibited the β_2 band. This indicated that Cgy_3^D also exhibited incomplete dominance to cgy_3^D when the classification system included the staining intensity relations.

"β_2" test for allelism. Crosses between P.I. 88.302-1 and P.I. 81.041-1 were made in order to test for allelism

Table 2. Observed and expected F_3 segregation ratios for the β_2, α^s and α'^s phenotypes occurring in families from two different crosses

Cross	F_2 phenotype	No. F_2 plants	Genetic ratio	Observed and (expected) progeny ratio	*P*
1. Century × P.I. 81.041-1	"β"	9	1 : 0 : 0 (β : β_2h : β_2)	89 : 0 : 0 (89) (0) (0)	–
	"β_2h"	17	1 : 3 (β : β_2h + β_2)	37 : 137 (43.5) (130.5)	> 0.25
			1 : 2 : 1 (β : β_2h : β_2)	37 : 78 : 59 (43.5) (87) (43.5)	> 0.01
	"β_2"	13	0 : 0 : 1 (β : β_2h : β_2)	5 : 11 : 113 (0) : (0) : (129)	–
2. Century × P.I. 54.608-1	"α"	7	1 : 0 : 0 (α : $\alpha\alpha^s$: α^s)	21 : 0 : 0 (21) (0) (0)	–
	"$\alpha^s\alpha$"	19	1 : 2 : 1 (α : $\alpha\alpha^s$: α^s)	17 : 26 : 14 (14.25) (28.5) (14.25)	> 0.69
	"α^s"	18	0 : 0 : 1 (α : $\alpha\alpha^s$: α^s)	4 : 1 : 48 (0) (0) (53)	–
	[a] "α'"	8	1 : 0 : 0 (α' : $\alpha'\alpha'^s$: α'^s)	–	–
	[a] "$\alpha'^s\alpha'$"	24	1 : 2 : 1 (α' : $\alpha'\alpha'^s$: α'^s)	15 : 40 : 16 (17.75) (35.5) (17.75)	> 0.68
	[a] "α'^s"	10	0 : 0 : 1 (α' : $\alpha'\alpha'^s$: α'^s)	–	–

[a] F_2 phenotype proposed on the basis of F_3 segregation ratio

between Cgy_3^D in P.I. 81.041-1 and the gene or genes which confer the β_2 phenotype in P.I. 88.302-1. Extracts of 80 F_2 seeds from this cross each produced a "β_2" gel pattern which was indistinguishable from the parental type (i.e. the staining intensity of the β_2 band was always similar to that of the β band and it migrated to the same location on the gel in each case; Table 1, cross 2). Furthermore, single gene segregation ratios for the "β_2" phenotype were observed in the F_2 progeny of P.I. 54.608-1 × P.I. 88.302-1 (Table 1, cross 5). This indicates that the "β_2" phenotype in P.I. 88.302-1 is conditioned by a gene which is probably identical or allelic to Cgy_3^D. It is also possible that this gene occupies a different locus, but is tightly linked to cgy_3^D.

Inheritance of the "α^s" phenotype. The "α^s" phenotype (Fig. 1 A, lane 6) exhibited an apparent upward "shift" in the position of the α band (α^s) in SDS-polyacrylamide gels. P.I. 54.608-1 was true-breeding for this aberration, and the trait remained unchanged after several generations of selfing in both field and greenhouse environments.

Extracts of F_1 seeds from reciprocal crosses between P.I. 54.608-1 and Century produced electrophoretic patterns with both normal and "shifted" bands ($\alpha\,\alpha^s$), and their staining intensity was approximately equal (Fig. 1 A, lane 5). Gel banding patterns from the F_2 seeds gave an approximate 1 : 2 : 1 segregation ratio for $\alpha : \alpha\,\alpha^s : \alpha^s$ and no reciprocal differences could be identified (Table 1, cross 3). These results were consistent with the hypothesis that the α^s band is governed by an allele which is codominant with the one that encodes the normal α subunit. To test this hypothesis, F_2 seeds of known phenotype were selfed and the electrophoretic banding patterns of their F_3 progeny were analysed (Table 2, cross 2). All seven F_2 plants which contained only α subunits were true breeding for this phenotype. F_3 seeds from the 19 F_2 plants that contained both α and α^s subunits exhibited either the α, $\alpha\alpha^s$, or α^s subunits in a ratio of approximately 1 : 2 : 1. All but five of the 54 F_3 seeds from the 18 F_2 plants which contained only α^s subunits also contained only α^s subunits. Although the five seeds which contained an α band could be the result of mutation or the action of modifier genes, we believe that they were simply due to incorrect classification of their parental F_2 seeds because of errors in reading the gels. Therefore, the observed progeny ratios are most simply explained by the segregation of two codominant alleles of a single gene. We propose the gene symbols Cgy_2^S and Cgy_2 be used to designate the alleles governing the "α^s" and "α" phenotypes, respectively.

Inheritance of the "α'^s" phenotype. The "α'^s" phenotype (Fig. 1 B, lane 4) was identified in P.I. 54.608-1 when urea was incorporated into the gel. This trait consists of an upwards shift of the α' band relative to the normal α' subunit and is analogous to that of the "α^s" phenotype in SDS gels without urea. P.I. 54.608-1 was also true-breeding for the α'^s band and, again, expression was not noticeably altered after several generations of growth in either greenhouse or field environments. The α^s band of P.I. 54.608-1 was not visible in the gels which contained urea, while the α'^s band was not evident in gels which did not contain urea.

Gel banding patterns of remnant seeds from the above crosses between Century and P.I. 54.608-1 were analyzed in order to investigate the inheritance of the α'^s band. F_1 seeds from either reciprocal cross contained both the normal α' and the shifted α'^s band (Fig. 1 B, lane 3). F_2 seeds segregated approximately 1 : 2 : 1 for $\alpha' : \alpha'\,\alpha'^s : \alpha'^s$ (Table 1, cross 3) and no reciprocal differences could be identified. These results were consistent with the hypothesis that the α'^s phenotype is governed by an allele which is codominant with the one that encodes the normal α' subunit. To test this hypothesis, three F_3 seeds from each of 42 F_2 plants of unknown phenotype were analyzed. As expected, when F_2 seeds were classified on the basis of F_3 segregation ratios, an approximate 1 : 2 : 1 ratio for $\alpha' : \alpha'\,\alpha'^s : \alpha'^s$ bands was obtained (8 : 24 : 10 $P > 0.60$). Furthermore, the summed F_3 data for the 24 segregating F_2 plants was also consistent with a 1 : 2 : 1 ratio (Table 2, cross 2).

The cultivar 'Keburi' is homozygous recessive for cgy_1, a null-allele for the α' subunit. When extracts of F_2 seeds from the cross Keburi × P.I. 54.608-1 were analyzed on SDS-polyacrylamide gels which contained urea, a close fit to a 3 : 1 ratio was obtained for presence : absence of α'^s (Table 1, cross 4). These and the above ratios from crosses between P.I. 54.608-1 and Century are most simply explained by allelism between the structural gene for α', the null-allele from Keburi, and a codominant gene from P.I. 54.608-1 which conditions the α'^s band. For this reason, we use the symbols $Cgy_1/Cgy_1^S/cgy_1$ to denote the three alleles.

Linkage results from trihybrid cross ("$\alpha^s\,\alpha'^s$" × "β_2"). Since P.I. 54.608-1 exhibits both the "α^s" and "α'^s" phenotypes and P.I. 88.302-1 exhibits the "β_2" phenotype, the cross between these two plant introductions provided segregation data and linkage information for genes which govern all three phenotypes. F_2 progeny ratios for the "α'^s", "α^s" and "β_2" phenotypes from P.I. 54.608-1 × P.I. 88.302-1 each followed closely the 1 : 2 : 1 ratio expected for a codominant or incompletely dominant gene (Table 1, cross 5). This confirmed the results of the above inheritance studies. Furthermore, no evidence for gene interaction was found.

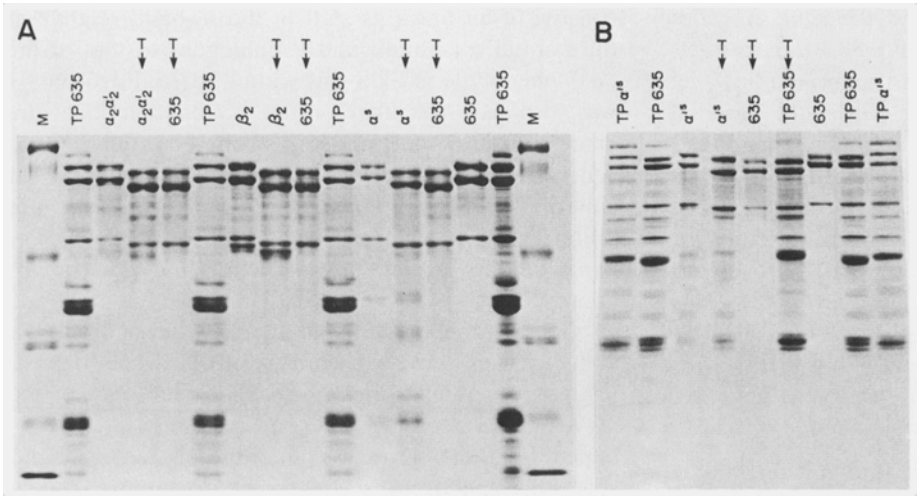

Fig. 2 A, B. SDS-PAGE of proteins from soybean seeds with variant phenotypes for 7S protein, before and after deglycosylation with TFMS. **A** SDS gel, **B** SDS-Urea gel; TP 635 = total seed protein from breeding line CX-635-1-1-1; 635 = 7S protein from breeding line CX-635-1-1-1; $\alpha_2\alpha'_2$ = 7S protein from seeds with "$\alpha_2\alpha'_2$" phenotype; β_2 = 7S protein from seeds with "β_2" phenotype; α^s = 7S protein from seeds with "α^s" phenotype; T = treated with TFMS; M = Molecular weight markers: phosphorylase b, 92,500; bovine serum albumin, 66,200; ovalbumin, 45,000; carbonic anhydrase, 31,000; soybean trypsin inhibitor, 21,500; lysozyme, 14,400; TPα'^s = Total seed protein from seeds with "α'^s" phenotype; 'α'^s = 7S protein from seeds with "α'^s" phenotype

The combined progeny ratio for the "α^s" and "β_2" phenotypes showed a significant departure from the expected 1 : 2 : 1 : 2 : 4 : 2 : 1 : 2 : 1 ratio for independent inheritance ($P < 0.005$). This departure was due to an overabundance of parental types and is indicative of linkage between the two genes which govern these traits. Combined progeny ratios for the "α'^s" and "β_2" or the "α'^s" and "α^s" phenotypes were both consistent with a model of independent inheritance.

From the above, the inheritance of the "α^s", "β_2" and "α'^s" phenotypes can be best described by the segregation of alleles from three respective loci: Cgy_2^S/Cgy_2 and Cgy_3^D/cgy_3^D, which are linked, and Cgy_1^S/Cgy_1, which segregates independently.

Inheritance of the "$\alpha_2\alpha'_2$" phenotype. The "$\alpha_2\alpha'_2$" phenotype exhibited doublet α' and α bands in SDS gels both with and without urea (Fig. 1 B, lane 5; Fig. 1 A, lane 7). Although P.I. 90.567-1 was true-breeding for this trait, considerable variation in relative staining intensity between normal and aberrant bands was noted between selfed generations. An additional band below the β subunit was also occasionally observed.

Extracts of F_1 seeds from the cross P.I. 90.567-1 × Century ("$\alpha_2\alpha'_2$" × normal) exhibited a normal banding pattern when analyzed on SDS gels. This indicated that the gene or genes which condition the doublet bands are recessive to those which code for a normal phenotype.

Additional genetic information for the "$\alpha_2\alpha'_2$" phenotype was obtained by analyzing the extracts of F_1 seeds from the cross P.I. 90.567-1 × P.I. 54.608-1 ("$\alpha_2\alpha'_2$" × "$\alpha^s\alpha'^s$") on SDS gels both with and without urea. In both cases, the characteristic doublets of the "$\alpha_2\alpha'_2$" phenotype were absent, confirming the recessive mode of inheritance for this trait. In gels without urea, the F_1 seeds contained a normal α band along with the expected codominantly-inherited α^s band from P.I. 54.608-1 (Fig. 1 A, lane 8). Similarly, a normal α' band was observed along with the codominantly inherited α'^s bands in gels with urea (Fig. 1 B, lane 6). These patterns are most simply explained by a model in which a *trans*-acting gene or genes from P.I. 54.608-1 encode enzymes which are altered in P.I. 90.567-1 and are required for processing of both "shifted" and normal subunits. A *trans* effect is further suggested by the independent inheritance of genes which encode the α and α' subunits (see above section), since both subunits exhibit the doublet phenotype simultaneously.

2 *Biochemical studies*

Analysis of deglycosylated protein. The standard techniques used to purify normal 7S proteins were also effective in purifying the variant proteins responsible for the "α^s", "α'^s", "β_2" and "$\alpha_2\alpha'_2$" phenotypes. The electrophoretic mobilities of all the subunits of the purified 7S proteins, before and after deglycosylation with TFMS, are shown in Fig. 2 A and B. All of the 7S subunits from both the normal and variant phenotypes migrated faster after deglycosylation than before. This result showed that all of the native subunits contained carbohydrate.

When the mutant and normal proteins were compared after deglycosylation, the α'^{s}, α^{s}, and β_2 variant subunits retained altered electrophoretic mobility compared with the equivalent normal subunits (Fig. 2 A and B). In other words, the α'^{s} and α^{s} bands are "shifted" slightly upwards compared with deglycosylated normal α' or α bands, and the "β_2" variant still contains an additional band below the deglycosylated β subunit. However, the opposite result was obtained for the variant peptides from the "$\alpha_2\alpha'_2$" phenotype. In this case the deglycosylated 7S proteins from the "$\alpha_2\alpha'_2$" phenotype and the normal phenotypes had identical electrophoretic mobilities (Fig. 2 A). These results suggest that the extra subunits in the "$\alpha_2\alpha'_2$" phenotype result from a change in the carbohydrate moieties of the proteins, whereas the altered subunits in the "α'^{s}", "α^{s}" and "β_2" phenotypes result from changes in the amino acid sequences of the proteins.

Discussion

In this study we have identified three genes which encode variant β-conglycinin subunits. Alleles for two of these genes are inherited in a codominant fashion, with the alternate allele coding for a normal subunit protein (Cgy_1/Cgy_1^{S}, α' subunit; Cgy_2/Cgy_2^{S}, α subunit). Cgy_1/Cgy_1^{S} was shown to be allelic to cgy_1, a previously described gene which conditions the absence of α' in cgy_1/cgy_1 genotypes (Kitamura et al. 1984). The third gene, which controls the appearance of an extra β electrophoretic band exhibited incomplete dominance (Cgy_3^{D}/cgy_3^{D}, β subunit). All three aberrant subunits differed from the normal in the protein portion of the molecule, as was indicated by their retention of altered electrophoretic mobility following deglycosylation of both normal and aberrant subunits. The most straightforward explanation for these results is that the mutations which produce the aberrant bands are in the structural genes for the α', α and β subunits. This explanation implies that there is only one functional coding sequence for the α structural gene, a single functional coding sequence for the α' subunit, and in soybean lines exhibiting the "β_2" phenotype, two or more functional sequences that encode β subunits. Our results also indicate that the α gene is tightly linked to one of the β genes, whereas the α' gene is located elsewhere in the genome since it segregates independently of the α and β genes.

Recent results from sequence analysis of a genomic library have indicated that the α' and α subunits are coded by only 1 or 2 genes, whereas the β subunit is clearly multigenic (J. Harada and R. B. Goldberg, personal communication). These data further show that a gene which encodes a β subunit is linked to one which probably encodes an α subunit. Both the gene number and linkage relationships for the α', α and β subunits deduced from these experiments are consistent with our genetic model.

β-Conglycinin subunits and the nucleotide sequences encoding them have been studied intensively. Considerable homology exists between α, α' and β sequences and this has been used as evidence to suggest that β-conglycinin subunits are the products of a multigene family (Schuler et al. 1982). It has further been suggested that each subunit may itself reflect a small sub-family of polypeptides. The latter conclusion is based on observations that purified β-conglycinin subunits can be separated into two or more components by isoelectric focusing (Thanh and Shibasaki 1977), as well as two-dimensional electrophoresis (Lei et al. 1983; Hu and Esen 1982; Ladin et al. 1984). The charge microheterogeneity would appear to reflect different coding regions, since the DNA sequences of four cDNA clones for α and α' subunits showed approximately 1% variation in sequence among clones that encoded the same subunit (Schuler et al. 1982). Although the suggestion that there are multiple coding sequences for each β-conglycinin subunit is consistent with the inheritance of the aberrant β subunit, this level of complexity does not fit with the model of single gene inheritance for the α and α' subunits.

Complex gene interactions may somehow account for the differences between our genetic data and the suggestion that there are multiple α and α' genes. For instance, a mutation in a *cis*-acting regulatory gene or region adjacent to a series of tandemly linked structural genes could be inherited in a single gene, codominant fashion. It is, however, difficult to imagine how a mutation of a regulatory gene or region of DNA would condition a simultaneous change in the primary structure of every subunit encoded within the cluster.

The shifted α and α' phenotypes were differentially affected by urea, suggesting that these subunits are not completely unfolded by the electrophoresis buffers and that the shifted mobilities actually reflect differences in three-dimensional structure. Accordingly, structural changes that give rise to the shifted phenotype need not reflect large alterations in the subunit primary structure but could arise from a change as small as one amino acid substitution. This explanation would also account for the apparent comigration between variant and normal gene products after a change in urea concentration (for example, comigration of α and α^{s} subunits in urea gels or α' and α'^{s} subunits in those that lack urea).

The recessive inheritance of the "$\alpha_2\alpha'_2$" phenotype is unusual. Genes encoding altered electrophoretic bands in legume storage proteins are typically inherited in a codominant manner (Brown et al. 1981; Casey 1982). Results of deglycosylation experiments indicate that the mutant glycoproteins differ from the normal in the carbohydrate portion of the molecule. The carbohydrate moieties of β-conglycinin are known to be of the high-mannose type (Yamauchi and Yamagishi 1979), and each moiety is linked to an asparagine

residue in the protein chain *via* a N-glycosidic bond (Yamauchi et al. 1975; 1976). The α and α' subunits are thought to contain two carbohydrate moieties per protein molecule, whereas the β subunit contains only one (Thanh and Shibasaki 1977). These glycoproteins are known to undergo extensive co- and post-translational modifications, although the exact steps between translation and packaging into protein bodies remain unclear. Sengupta et al. (1981) suggest a sequence of glycosylation, deglycosylation and proteolysis events and the formation of higher molecular weight intermediates.

Thus, the mutation may either affect the structure of enzymes involved in glycosylation or alter the concentration of glycosylation enzymes during seed development. The *trans*-acting gene or genes of P.I. 90.507-1 may be a useful biochemical tool for investigating these events.

Acknowledgements. The authors express their appreciation to Ms. C.H. Murphy and Ms. P. Gunaratne for their skillful technical assistance.

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