GENETIC VARIABILITY AND INHERITANCE OF METHIONINE-RICH 2S ALBUMINS IN SUNFLOWER

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Abstract

Protein 2S albumin fractions were isolated from seeds of 103 sunflower accessions and analysed by SDS-PAGE, IEF and RP-HPLC. Two methionine-rich albumins, SFA7 and SFA8, showed no differences in mobility on SDS-PAGE gels. However, their levels demonstrated wide variation among different genotypes in relation to each other and as proportions of the total albumin fraction. A variant form of SFA8 was identified which differed from the normal SFA8 in its pI (6.5 compared to 6.0) and mobility in SDS-PAGE. *N*-terminal sequences of both variant and the major forms of SFA7 were identical to that reported previously for the normal form of SFA8 from the cultivar Hysun (Kortt et al., 1991) indicating their structural relatedness. Analysis of segregation in the F2 of the cross between lines VIR 130 (variant SFA8) and VIR 104 (normal SFA8) showed that the normal and variant forms of SFA8 are encoded by different alleles at a single Mendelian locus. The levels of SFA7 and SFA8 in the seeds of parental lines, F1 hybrid and individual F2 seeds classified from SDS-PAGE and IEF as homozygous for normal SFA8, homozygous for variant SFA8 and heterozygous were determined by RP-HPLC. The proportions of SFA7 and SFA8 were inversely correlated among individual F2 seeds. The results suggest that the amounts and proportions of SFA7 and SFA8 are determined by genetic factors in addition to sulphur.

Introduction

Sunflower (*Helianthus annuus* L.) seeds contain two major groups of storage proteins, 11S globulins (called helianthinin) and 2S albumins, present in a ratio of about 2:1 (Mazhar et al., 1998). The 2S albumins belong to a widely distributed family of seed proteins (Shewry and Pandya, 1999) and comprise single subunits with *Mr* ranging from about 10,000 to 18,000 (Kortt and Caldwell, 1990; Anisimova et al., 1995). Kortt and Caldwell (1990) purified eight sunflower albumins by RP-HPLC and showed that the two components with the longest

retention times, which they called SFA7 and SFA8, each contained about 15 mol% methionine. SFA8 was the quantitatively major component of the two and has subsequently been studied in some detail, including determination of its amino acid sequence (Kortt et al., 1991), disulphide structure (Egorov et al., 1996), folding and structure (Pandya et al., 1999, 2000) and the isolation of a cDNA clone (Kortt et al., 1991) which has been used to increase the methionine content of transgenic lupin seeds (Molvig et al., 1997). Recent studies have shown IgE-binding capacity of a methionine-rich protein from sunflower seed (Kelly et al., 1999; 2000). However, nothing further has been reported on the characteristics of SFA7. We have therefore carried out detailed studies of the relationships, polymorphism and inheritance of these two methionine-rich albumins.

Materials and Methods

The materials examined comprised 103 accessions of sunflower seeds obtained from VIR World Collection (St. Petersburg, Russia), University of Giessen (Germany) and University of Birmingham (United Kingdom). A modified acetone precipitation method (Kortt and Caldwell, 1990) was used to isolate albumin fractions from parts of seeds (up to 2–4 mg). The precipitated 2S albumins were dissolved in water and then freeze dried or directly analysed using high performance liquid chromatography (RP-HPLC), sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) or isoelectric focusing (IEF).

Results and Discussion

Albumin fractions from 103 accessions of sunflower seeds, comprising 65 inbred lines, four cultivars, 19 interspecific hybrids and 15 wild species, were screened by SDS-PAGE to identify differences in their polypeptide compositions, particularly in the amount or mobility of the methionine-rich proteins SFA7 and SFA8. The two methionine-rich albumins, SFA7 and SFA8, co-migrated to give a single band. This band varied in staining intensity, being low in genotypes poor in both albumins and high in genotypes rich in either SFA7 or in SFA8. Minor differences in the mobility of the SFA7 and SFA8 bands were observed, with a "normal" type typified by the line VIR 104 and a slightly slower "variant" type typified by the line VIR 130. This variant form was identified in only five of the 65 lines, all of which were derived from the German accession K2266 (lines VIR 664, 265, 130) or the Polish line L1648/1 (lines VIR 666 and 676). RP-HPLC of the fractions from lines VIR 130 and VIR 104 showed that both were rich in SFA8 but low in SFA7 (Figure 1a, c), indicating that the polymorphism observed on SDS-PAGE resulted from differences in the properties of SFA8. However, no difference in the retention times of the two forms was observed, either when mixed (not shown) or in the F1 hybrid (Figure 1b). In contrast, IEF gave clear separation of the two forms with the normal form having a pI of about 6.0 and the variant form a pI of about 6.5.

To determine the inheritance of the two forms of SFA8 a cross was made between VIR 104 (normal SFA8) and VIR 130 (variant SFA8) and the F1 and F2 progeny analysed by SDS-PAGE and IEF. The two forms were expressed codominantly in the F1 seed while the ratio of phenotypic classes in a sample of 89 F2 seeds fitted a 1:2:1 ratio (27 VIR 130; 42 heterozygotes; 20 VIR 104 (χ-square= 1.355, $P>0.05$). Small amounts of the normal form of SFA8 were also observed in seeds homozygous for the variant form but no trace of the variant form was observed in normal homozygotes.

To confirm the identity and relatedness of the various methionine-rich albumins, *N*-terminal amino acid sequences were determined for the variant form of SFA8 blotted from an IEF separation of albumins from the line VIR 130 and of SFA7 purified from the hybrid cultivar Sunbred 246 by RP-HPLC. The *N*-terminal sequences of both the variant form of SFA8 and the major form of SFA7 were identical to that reported previously for the normal form of SFA8 from the cultivar Hysun 33 (Kortt et al*.,* 1991): Pro.Tyr.Gly.Arg.Gly.Arg. Thr.Glu.Ser.Gly.

Comparison of the various genotypes showed a wide range of variation in the levels of SFA7 and SFA8, in relation to each other and as proportions of the total albumin fraction. In order to determine the genetic control of the quantitative variation in SFA7 and SFA8, 48 individual seeds from the cross between VIR 130 and VIR 104 were analysed by RP-HPLC (Figures 1 and 2).

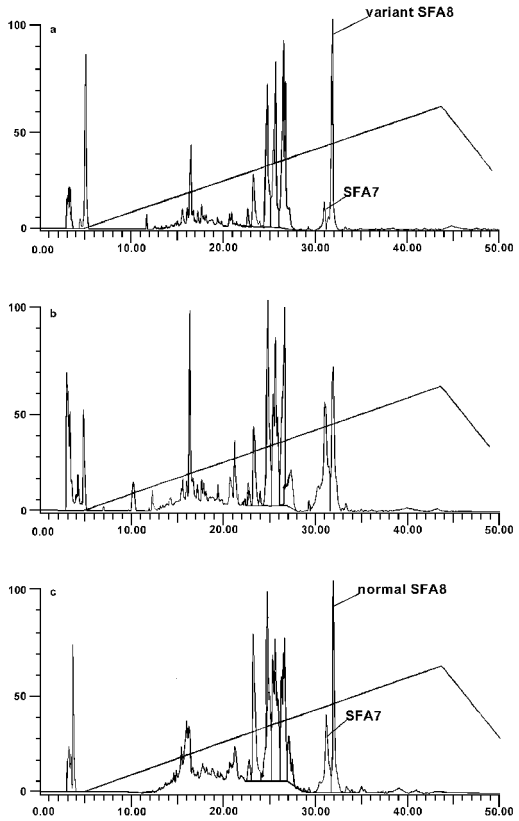

Figure 1. RP-HPLC analyses of 2S albumin fractions from the inbred lines VIR130 (a), VIR104 (c) and F1 hybrid (VIR130×VIR104) (b).

The parents contained similar combined proportions of SFA7 and SFA8 (22.7 and 22.6%, respectively) but differed in that VIR 130 contained 3.7% SFA7 and 19.0% SFA8 whereas VIR 104 contained 9.9% SFA7 and 12.8% SFA8 (Table 1). The F1 hybrid contained a higher total

amount of SFA7 + 8 (32.0%) which was largely accounted for by a high proportion of SFA7 (18.4%). The F2 seeds from the VIR 130 x VIR 104 cross were classified by IEF and SDS-PAGE into three classes: homozygous for normal SFA8 (VIR104 type), homozygous for variant SFA8 (VIR 130 type) and heterozygous (F1 type). There was little difference between the mean levels of SFA7+8 in the three phenotypic classes, all containing about 18-19% (Table 1).

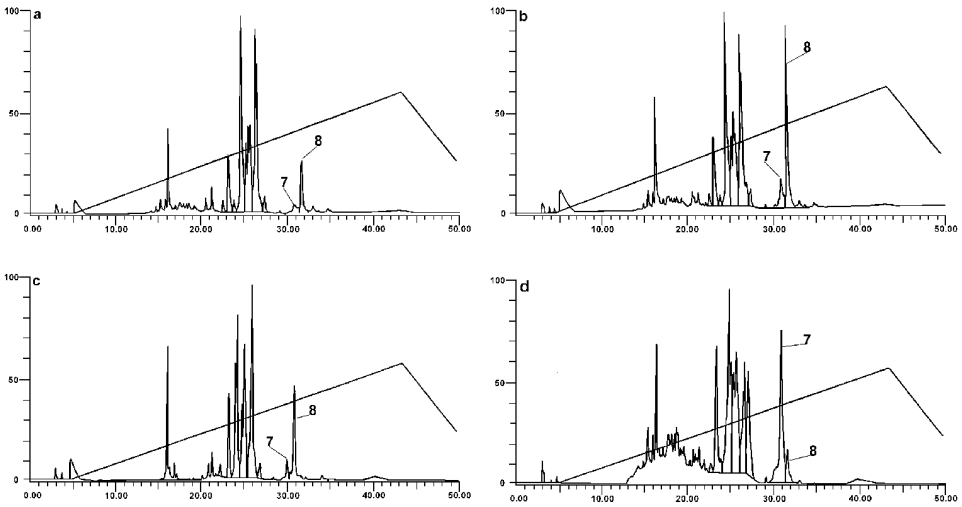

Figure 2. RP-HPLC profiles of 2S albumins from individual F2 seeds which were classified on the basis of IEF and SDS-PAGE analyses as VIR 130-type (c), VIR 104-type (b), F1-type (a, d).

Table 1. Proportions of SFA7 and SFA8, expressed as % total 2S albumins, in parental, F1 and F2 seeds from the cross VIR 130 × VIR 104.

Protein	Level	Genotype					
		Parental		F1	F2		
		VIR 130	VIR 104		VIR 130	F1	VIR 104
SFA7	max	–	–	–	7.813	18.477	7.930
	min	–	–	–	2.793	2.828	3.856
	mean	3.694	9.858	18.431	4.514	7.530	5.925
SFA8	max	–	–	–	18.653	15.589	15.745
	min	–	–	–	9.159	3.530	7.389
	mean	19.043	12.771	13.569	14.140	10.608	12.519
SFA7+8	max	–	–	–	24.287	24.113	19.727
	min	–	–	–	10.312	10.526	12.121
	mean	22.737	22.629	32.000	18.633	18.138	18.444
Sample size		–	–	–	14	25	9

This is slightly lower than the levels present in the two parental lines (≅22.6 and 22.7%) and considerably lower than that present in the F1 hybrid (32%). However, more variation was observed between the individual seeds, from about 10-20%.

The ratio of SFA7 to SFA8 also varied considerably, being highest in the VIR 104-type and heterozygous seeds. This is summarised in Table 2, which shows that the amount of SFA7 exceeded that of SFA8 in six seeds (all were heterozygotes) but not in any VIR 130- or VIR 104-type seeds.

Table 2. Frequencies of occurrence (%) of F2 seeds with different proportions of SFA7 and SFA8 from the cross VIR 130 × VIR 104.

Proportion of SFA7 (as % SFA8)	Total	Phenotypic class		
		VIR130	F1	VIR104
Low SFA7 (≤30%)	7	42.9	0	11.1
Intermediate SFA7 (31–50%)	20	50.0	36.0	44.4
High SFA7 (51–100%)	15	7.1	40.0	44.4
Very High SFA7 (110–500%)	6	0	24.0	0

Statistical analysis showed a significant (at the significance level P=0.01) inverse correlation between the proportions of SFA7 and SFA8 in the F2 seeds. However, the correlation coefficient (*r*) was low, being –0.56 for all 48 seeds examined.

Two major forms of methionine-rich albumin are present in sunflower seed, called SFA7 and SFA8. These are structurally related and both also occur in allelic 'normal' and 'variant' forms which differ in pI on IEF or mobility on SDS-PAGE. Genetic analysis indicates that they are encoded by a single locus which is not linked to the locus (or loci) encoding the majority of the 2S albumins, which are not methionine-rich. However, it is currently not possible to rule out the possibility that SFA7 is a modified form of SFA8, or vice versa, particularly since 2S albumins of all species are known to undergo post-translational processing (Shewry and Pandya, 1999).

It could be expected that the synthesis of methionine-rich proteins such as SFA7 and 8 would depend on the availability of sufficient sulphur to support their high contents of methionine. This occurred when SFA8 was expressed in seeds of transgenic lupin (Molvig et. al., 1997). Although an increase in total seed methionine occurred, this was at the expense of free sulphate and, to a lesser extent, cysteine, with no increase in total seed sulphur. Although the proportions of SFA7 and SFA8 were inversely correlated in the F2 population of a cross, their combined proportions varied from about 10 to 24%, with about 22% in each parent and 32% in the F1 seeds. This indicates that the amounts and proportions of these proteins are not determined solely by competition for sulphur but also by genetic factors. Such a genetic factor, Zpr 10(22), responsible for accumulation of methionine-rich zein storage protein 10-K in maize seeds was identified by classical genetic analysis (Benner et al., 1989). A significant heterotic effect on the level of SFA7 in the F1 seeds can be explained as a result of interallelic complementation which has been widely described for biochemical characters (mainly isoenzymes) in plant and animal kingdoms (Fincham, 1966).

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