Differential effect of sulphur deficiency on the composition of the aminoacyl-tRNA and free amino acid pools of the developing pea seed

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Both aminoacyl-tRNA and free amino acids were extracted from cotyledons of *Pisum sativum* L., cv. Greenfeast grown under either sulphur-deficient or control conditions, at a stage of active synthesis and accumulation of storage proteins. Sulphur deficiency severely perturbed the composition of the free amino acid pool, causing a marked decrease in the relative level of cysteine but no change in methionine, as well as large increases in arginine, asparagine and other amino acids. On the other hand, the proportions of both sulphur and non-sulphur amino acids in the aminoacyl-tRNA pool were unaltered. This lack of a specific effect of the deficiency on the sulphur amino acids of the aminoacyl pool makes it unlikely that the altered pattern of storage protein synthesis and accumulation is due to unavailability of these amino acids.

Aminoacyl-tRNA *Free amino acid* *S deficiency* *Seed development*
Pea Pisum sativum

1. INTRODUCTION

Several groups [1–3] have shown that when plants are grown under sub-optimal sulphur supply, marked changes occur in the proportions of proteins deposited in the developing seeds. In the case of the pea, *Pisum sativum* L., a large decrease in legumin and some of the major albumins of the seed is accompanied by an increase in the vicilin fraction [3] (H.E. Schroeder, personal communication). The S amino acid content of the former proteins is significantly greater than that of vicilin [4–7]. These changes in the S-deficient seed could conceivably arise as a result of decreased availability of S amino acids for protein synthesis, leading to preferential synthesis of proteins less rich in S amino acids. I have therefore investigated the effect of S-deficiency not only on the free amino acid pool, but also on the pool of amino acids covalently linked to tRNA, since the latter are the immediate substrates for polypeptide synthesis.

2. MATERIALS AND METHODS

Pisum sativum L. cv. Greenfeast, line 086 was grown at 20°C with a 16 h photoperiod, in 1:1 sand–perlite; the nutrient for S-deficient and control plants contained 0.05 mM and 1 mM sulphate, respectively, as in [3]. Several S-deficient and control pods were taken from the lower flowering nodes 22 days after flowering, and the cotyledons were quickly dissected out and dropped into liquid N_2.

Chemicals were obtained from the following sources: bentonite from Sigma, dithiothreitol (DTT) from Calbiochem, Sephadex G-25 from Pharmacia, DEAE-cellulose (DE-23) from Whatman, ^{14}C-labelled amino acids and [^{3}H]dansyl chloride from Amersham, and iodoacetic acid from Merck. Triethylamine (from BDH) was purified by distillation from phenyl isocyanate.

Isolation of aminoacyl-tRNA was carried out at 0–5°C unless otherwise stated. After powdering the frozen cotyledons in a mortar under liquid N_2

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they were homogenised, while thawing, in an emulsion of equal volumes of phenol–*m*-cresol–water (50:7:10, by vol.) and 0.3 M acetate, 0.5 mM EDTA, 1% bentonite, 1% SDS and 10 mM DTT (pH 4.5). After centrifugation, the phenol phase was re-extracted twice with 0.3 M acetate, 0.5 mM EDTA and 5 mM DTT (pH 4.5). Total nucleic acid was precipitated at −20°C overnight by the addition of 2 vol. of ethanol to the combined aqueous phases. The pellet recovered by centrifugation was washed by resuspension in 70% ethanol, 0.1 M acetate, 1 mM DTT (pH 4.5) and then dissolved in 1 mM Na_2EDTA, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT ('EMS buffer'). After centrifuging down a small insoluble residue, any remaining free amino acids were removed by gel filtration on a Sephadex G-25 column in EMS buffer. Aminoacyl-tRNA (and other nucleic acid present in the preparation) was collected by adsorption to a small DEAE-cellulose column (bicarbonate form), packed over a disc of Miracloth (Chicopee Mills) in a syringe barrel equipped with a needle. After blowing out the interstitial liquid with a stream of N_2, the needle was blocked and the column bed resuspended in 1 M triethylammonium bicarbonate buffer (pH 8.5), to which a mixture of [^{14}C]amino acids had been added. The stoppered column was then incubated at 37°C for 1 h, after which the interstitial buffer, containing both the amino acids stripped from the tRNA and the [^{14}C]amino acids, was blown out as before and collected. After removal of the buffer by rotary evaporation at 40°C, the residue was dissolved in 0.2 M $NaHCO_3$. Cyst(e)ine was reduced and carboxymethylated by treating the solution with 2 mM DTT (final concentration) for 30 min at room temperature, followed by 5 mM iodoacetate (for 30 min in the dark) and finally by 5 mM mercaptoethanol. The solution was again taken to dryness and dissolved in water. Derivatisation with [^{3}H]dansyl chloride, thin-layer chromatography and amino acid quantitation were carried out as in [8].

The tRNA content of the gel-filtered nucleic acid preparation was determined by electrophoresis in 3% and 4% polyacrylamide gels in Tris–acetate–EDTA–SDS [9], followed by scanning at 260 nm.

The free amino acid pool was extracted from cotyledons with 6.7% trichloroacetic acid and analysed by dual-label dansylation as in [8].

3. RESULTS AND DISCUSSION

The S-deficient plants used in this study clearly showed the symptoms of lowered chlorophyll content, progressive stunting towards the apex and markedly reduced total seed S described in [3]. Pods were taken 22 days after flowering. At this time the major proteins being synthesised in the seeds of control plants are legumin and vicilin, together with two albumins having polypeptides of M_r 22000 and 8000, respectively (unpublished), whereas in S-deficient seeds the synthesis of all these proteins except vicilin is greatly reduced [10], (H.E. Schroeder, personal communication).

S-deficient cotyledons had 70–90% of the fresh weight of control cotyledons, but contained only 30–40% of the tRNA. Based on summation of the data in table 1 the aminoacyl-tRNA pool of 2.1 nmol amino acids/S-deficient cotyledon and 6.4 nmol/control cotyledon was smaller (by a factor of 10^3–10^4) than the free amino acid pool (22.8 and 5.1 μmol, respectively).

Analysis of the free amino acid pool (table 1) revealed marked effects of S-deficiency. Conspicuous among these were a depression of cysteine level to 20% of the control, big increases in arginine and asparagine, and considerable rises in other amino acids; the total free amino acid pool underwent a 4-fold expansion. These changes are in many respects similar to those found earlier in leaves and roots of other species under S-deficiency [11,12]; their metabolic basis is not known.

In contrast to this perturbation of composition of the free amino acid pool, there was no significant effect of S-deficiency on the relative levels of any of the components of the aminoacyl pool (table 1), even though this pool had contracted to 1/3 of the control. Particularly noteworthy is the lack of effect on cysteine or methionine.

These results strongly suggest that the reduced synthesis of the more S-rich seed proteins, such as legumin, is not due to unavailability of the S amino acids for the protein-synthesising system. In recent work from this laboratory [10] it was found that the legumin mRNA level is greatly reduced in S-deficient cotyledons and that it quickly recovers when S-deficient plants are restored to adequate S-nutrition. Taken together with the present results showing a lack of effect of S-deficiency on the

Table 1

Composition of the aminoacyl and free amino acid pools in sulphur-deficient (−S) and control (C) cotyledons

	Aminoacyl pool pmol/unit tRNA			Free amino acid pool μmol/g fresh wt		
	−S	C	−S/C	−S	C	−S/C
Alanine	255	266	1.0	4.6	0.67	6.9
Arginine	162	156	1.0	67	0.83	81
Asparagine	112	93	1.2	8.4	0.48	18
Aspartate	160	144	1.1	1.8	4.8	0.38
Cysteine	34	40	0.9	0.053	0.32	0.17
Glutamate	435	447	1.0	10.8	4.7	2.3
Glutamine	134	153	0.9	0.78	2.4	0.33
Glycine	378	389	1.0	0.89	0.40	2.2
Histidine	135	197	0.7	0.66	0.24	2.8
Isoleucine	89	123	0.7	0.45	0.104	2.3
Leucine	213	214	1.0	0.25	0.099	2.5
Lysine	150	162	0.9	0.48	0.100	2.1
Methionine	69	69	1.0	0.076	0.068	1.1
Phenylalanine	85	97	0.9	0.17	0.105	1.6
Proline	114	164	0.7	0.095	0.071	1.3
Serine	239	343	0.7	1.16	0.56	2.1
Threonine	166	165	1.0	15.2	1.07	14
Tryptophan	101	125	0.8	0.112	0.113	1.0
Tyrosine	126	90	1.4	0.19	0.14	1.4
Valine	119	131	0.9	0.73	0.24	3.0

The data for each pool represent the mean of 2 expt

composition of the aminoacyl pool, this makes it probable that mRNA levels play the major role in regulating legumin synthesis in S-deficient cotyledons. However, there may be subtle changes in the tRNA population, or in the extent of charging of particular tRNAs, that play a subsidiary role in regulating the expression of the storage protein genes.

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