Phytochemistry, 1981, Vol. 20, pp. 21–23. © Pergamon Press Ltd. Printed in England. 0031-9422/81/0101-0021 $02.00/0

VARIATION IN AMINO ACID COMPOSITION OF LEGUMIN FROM *PISUM*

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(*Revised received* 23 *April* 1980)

Key Word Index—*Pisum*; Leguminosae; pea; amino acid composition; seed storage proteins; legumin.

Abstract—Legumin has been purified from a wide range of *Pisum* types and the amino acid compositions of the variant legumins have been determined. The observed variability in the methionine and cysteine contents of the variants is discussed in relation to the potential for selecting *Pisum* genotypes with improved seed protein quality.

INTRODUCTION

The protein of pea (*Pisum*) seeds, in common with that of many legume grains, is limited in its nutritional suitability for man and other monogastric animals by its low content of the sulphur-containing essential amino acids methionine and cysteine. This deficiency reflects the fact that the storage proteins of *Pisum* seeds, the water-insoluble globulins, contain little methionine and cysteine. *Pisum* seed globulins consist of two major species, vicilin and legumin [1], of which the latter contains more cysteine and methionine than the former [2–4]; it has thus been considered that an increase in the proportion of legumin to vicilin would be a desirable objective in the breeding of *Pisum* seeds with protein of increased nutritional quality.

Smartt *et al.* [5] have discussed a number of potential ways of improving the protein quality of grain legume seeds by breeding and concluded that such a process could proceed by selection of protein types, from a polymorphic series, having the most desirable methionine and cysteine contents and by selection for genotypes which produce maximal amounts of those proteins with desirable methionine/cysteine contents. We have previously purified legumin from a wide range of *Pisum* types and demonstrated the existence of considerable genetic variability in the types and numbers of the subunits which constitute the oligomeric legumin molecule [6]. In this article we report the extent to which this polymorphism is reflected in the methionine/cysteine content of the legumin variants and discuss the significance of such variability in the context of breeding for increased protein quality in *Pisum* seeds.

RESULTS AND DISCUSSION

The *Pisum* genotypes were originally selected on the basis of their legumin subunit structure, but also covered a range of forms including *P. fulvum*, and primitive and modern cultivars of *P. sativum* (see ref. [6]).

By all the criteria of purity the legumin preparations from these genotypes were uncontaminated, which, taking into account the limits of the methods involved, means that all samples were at least 97% pure. The legumins displayed the same genotype-specific subunit patterns on SDS-gel electrophoresis as described before [6].

The amino acid compositions of the six legumin variants (Table 1) showed the high levels of glutamate, aspartate and arginine and the low levels of cysteine and methionine characteristic of the molecule. There were no large differences in amino acid composition between the various legumins, although those from the *P. fulvum* genotypes (JI 224 Br and JI 849) showed some slight compositional differences: both contained reduced amounts of arginine, while JI 224 Br legumin also contained less aspartate than others. The significant figures in terms of seed protein quality, however, are those for cysteine and methionine: the mean values for the former ranged from 0.81 to 1.21 mol% and for the latter, from 0.28 to 0.72 mol%; the range of total sulphur-containing amino acids is 1.09–1.75 mol%. This is equivalent to a range of 1.01–1.78 g %, with a 'mean' methionine/cysteine content of 1.4 g %.

There are few reports in the literature of genotypic variability in the amino acid composition of *Pisum* seed proteins. Jackson *et al.* [2], Goa and Strid [7] and Grant and Lawrence [3] have reported compositions for vicilin, legumin and albumins, but from single genotypes. Hurich *et al.* [8] have described the amino acid compositions of albumins and the G1 and G2 fractions [9] from a range of *Pisum* genotypes; whilst the G1 fraction from *Pisum* is vicilin-like, the G2 fraction does not represent a pure legumin preparation (R. Casey, unpublished results) and its amino acid composition should be treated with some caution.

What, then, is the significance of the observed variability in legumin methionine/cysteine content and variation in legumin content of the whole protein in terms of seed protein quality? This can be calculated using the range of legumin compositions reported above, the range of albumin compositions listed elsewhere [8, 10], the known compositions for vicilin [2, 3], the reported range of legumin and albumin contents [11, 12] and by making the assumption that any increase in legumin is compensated by a relative decrease in vicilin. The

Table 1. The amino acid compositions of legumins from a range of *Pisum* genotypes

Amino acid	Genotype					
	JI 224 Br	JI 181	JI 227	JI 849	JI 184	JI 407
	Molar ratio (mol %)					
Lys	4.81 (0.06)	4.42 (0.04)	4.44 (0.01)	4.34 (0.04)	4.38 (0.05)	4.73 (0.30)
His	2.75 (0.01)	2.82 (0.03)	2.16 (0.01)	2.61 (0.03)	2.37 (0.08)	2.68 (0.13)
Arg	8.91 (0.03)	9.67 (0.09)	9.86 (0.03)	8.39 (0.07)	9.64 (0.38)	9.47 (0.24)
Asp	8.96 (0.01)	11.17 (0.02)	10.75 (0.20)	11.87 (0.01)	11.56 (0.06)	11.58 (0.13)
Thr	3.27 (0.13)	3.52 (0.04)	3.46 (0.03)	3.64 (0.01)	3.63 (0.38)	3.44 (0.04)
Ser	6.03 (0.06)	6.64 (0.07)	6.34 (0.06)	5.99 (0.02)	6.25 (0.19)	6.05 (0.08)
Glu	20.42 (0.15)	19.43 (0.05)	19.59 (0.41)	19.59 (0.03)	19.60 (0.04)	19.13 (0.30)
Pro	5.82 (0.47)	5.34 (0.01)	5.30 (0.13)	5.45 (0.13)	5.42 (0.03)	5.47 (0.14)
Gly	7.23 (0.06)	7.08 (0.01)	6.77 (0.05)	7.45 (0.02)	6.97 (0.05)	7.14 (0.11)
Ala	5.35 (0.08)	5.77 (0.01)	5.92 (0.01)	5.69 (0.01)	5.91 (0.04)	5.97 (0.16)
½-Cys*	0.81 (0.01)	1.08 (0.01)	1.12 (0.05)	1.21 (0.02)	1.16 (0.08)	1.03 (0.03)
Val	5.81 (0.05)	5.43 (0.01)	5.81 (0.12)	5.55 (0.02)	4.94 (0.04)	5.01 (0.03)
Met*	0.28 (0.01)	0.41 (0.01)	0.45 (0.01)	0.39 (0.03)	0.59 (0.04)	0.72 (0.06)
Ile	4.48 (0.06)	4.12 (0.03)	4.40 (0.09)	4.35 (0.04)	4.01 (0.03)	4.00 (0.03)
Leu	7.34 (0.35)	7.58 (0.08)	7.65 (0.16)	7.47 (0.02)	7.49 (0.04)	7.54 (0.08)
Tyr	2.42 (0.10)	2.39 (0.01)	2.46 (0.04)	2.29 (0.08)	2.30 (0.03)	2.37 (0.03)
Phe	3.34 (0.30)	3.14 (0.03)	3.56 (0.02)	3.76 (0.03)	3.82 (0.03)	3.70 (0.03)

Values are expressed as mean mol % with s.e. on the mean (for 2–4 determinations) in parentheses.
* Determined on performic-oxidized protein.

calculation shows that if the legumin contains the 'average' 1.4 g % methionine + cysteine and constitutes 50% of the total protein, the latter would have a methionine/cysteine content of *ca* 1.9 g %; a similar calculation with a legumin containing 1.8 g % methionine + cysteine realises a whole protein methionine/cysteine content of *ca* 2.2 g %, a figure which falls in the middle part of the range reported elsewhere for peas [13, 14]. Although this figure is subject to considerable uncertainty, since the calculation is made with incomplete information on the qualitative and quantitative variability of albumins, vicilins and other proteins, the magnitude of the increase does illustrate the desirability of breeding for increased levels of better quality legumin in pea seeds. It is, however, not sufficient to bring the composition of the whole protein to that of the F.A.O. 'standard protein' (*ca* 3.5 g %; ref. [15]); to achieve this an increase in the amounts of other proteins—possibly specific albumins [8, 10] or vicilin-3 (ref. [16])—would be desirable in addition to elevated quantities of high quality legumin.

The range of variation in methionine/cysteine content reported here is equivalent to *ca* two cysteine residues and three methionine residues per ($\alpha + \beta$) subunit of combined MW *ca* 60×10^3 [17–19]. Because legumin is usually composed of multiple α- and β-subunits [6, 19–22] (which may be the products of multiple, possibly duplicated, α- and β-loci [6, 23, 24]), the effect of any point mutation to a methionine or cysteine residue will be effectively 'diluted' by the presence of other homologous, non-mutated subunits [24] if the duplication process preceded the mutation; if, however, mutation preceeds duplication there will be no such 'dilution' effect.

EXPERIMENTAL

The *Pisum* genotypes used in this study (JI 181, 184, 224 Br, 227, 407 and 849) have been described elsewhere [6]. Mature, dry seed from greenhouse-grown plants was used in all cases and testas and embryonic axes were removed prior to protein extraction.

Legumin was purified from pea seed globulins by sequential $(NH_4)_2SO_4$ fractionation [18], conventional zonal isoelectric precipitation [19], gradient zonal isoelectric precipitation [21], DEAE-cellulose chromatography [18] and hydroxyapatite chromatography [21]; the final legumin prepn was dialysed extensively against dist., deionized H_2O and freeze-dried prior to amino acid analysis.

Legumin purity was determined by analytical ultracentrifugation, end-group analysis by dansylation, cellulose acetate electrophoresis and SDS-polyacrylamide gel electrophoresis, all as previously described [6, 18].

For amino acid analysis [25], purified legumin was hydrolysed for 24 hr in constant-boiling HCl. Values for threonine and serine were corrected by +5% and +10% respectively, for hydrolytic losses [26]. Cysteine and methionine were determined after performic acid oxidation [27]. From 2 to 4 determinations were carried out on all oxidized and non-oxidized samples.

Acknowledgements—We are very grateful to Miss E. Sanger and Mr. R. Francis for excellent technical assistance.

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