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SHORT REPORTS

HOMOLOGY OF BASIC SUBUNITS OF LEGUMIN FROM *GLYCINE MAX* AND *VICIA FABA*

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Key Word Index—*Vicia faba*; Vicieae; *Glycine max*; Glycineae; Leguminosae; amino acid sequence; basic subunits; legumin; glycinin; protein homology.

INTRODUCTION

Derbyshire *et al.* [1] have suggested that the 11/12S storage protein, legumin, is widely distributed in the Leguminosae. On the other hand, immunological cross reactivity studies have led Dudman and Millerd [2] and Millerd [3] to suggest that a protein homologous to the legumin of the Vicieae is absent in members of the tribe Glycineae (Phaseoleae) including soyabean. The answer to this question has awaited the determination of complete or partial amino acid sequences of these proteins from a member of the two tribes and in this paper, *N*-terminal partial sequences of the basic subunits of glycinin of soyabean and legumin of *Vicia* are presented.

RESULTS AND DISCUSSION

Vicia faba and *Glycine max* are members of the tribes Vicieae and Glycineae, respectively. Both glycinin from soyabean and legumin from broad beans share many structural features including, similar MWs, number and MW of subunits and possession of di-sulphide-bonded acidic and basic subunits. Table 1 shows the *N*-terminal amino acid sequence of the basic subunits of glycinin of soyabean and legumin of *Vicia*. A comparison shows that there is considerable similarity in the sequence, approximately two thirds of residue positions having the same apparent on SDS gels. Even so, it is also clear that the by chance and is unlikely to be a result of convergent evolution although in the absence of a function/structure analysis, it is not possible to be categorical. However, the most likely explanation is that these are two homologous proteins.

The basic subunits of both legumin and glycinin demonstrate appreciable heterogeneity when examined either by SDS gel electrophoresis [4] or by isoelectrofocusing [5, 6]. As this heterogeneity is not fully evident in the amino acid sequence so far determined, it implies that there must be regions of the remaining subunit structures which are far less conserved (i.e. contain more radical amino acid substitutions) than the *N*-terminal fragments. Changes in charge and hydrophobicity afforded by non-conservative amino acid substitutions would account for the multiplicity of species observed on isoelectrofocusing and also, through reduced or increased binding of detergent, for the different MW species apparent on SDS gels. Even so, it is also clear that the different basic subunits of each protein share so many residues in common as to be paralogous (homologous).

Recently [7] the subunit structure of the seed globulin of a *Cucurbita* sp. has been described. The molecule has several subunits of 36000 and 22000 daltons linked by disulphide bonds. The 36000 and 22000 subunits are acidic and basic, respectively. Both legumin and glycinin show very similar structural features [1]. Furthermore the Japanese workers determined the *N*-terminal sequence of both the two separated basic subunits as Gly-Leu-Glu(Asp)-Gly-Thr-Ile. These amino acids are identical to one or other of the equivalent legumin or glycinin *N*-terminal residues suggesting that protein homology can be extended to another family, the Cucurbitaceae, also in the Dicotyledonae. On strictly logical grounds it could be suggested that the name glycinin should be discontinued and replaced by legumin. However, since the former term is firmly establshed by usage, such a change might lead to difficulties.

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Table 1. *N*-Terminal amino acid sequence of the basic subunits of the 11/12S storage protein of *Vicia faba* and *Glycine max*

	1	5	10	15	20	25	30
Vicia faba (Legumin)	G L E E T $\frac{V^2}{I^1}$ C T $\frac{V^2}{A^1}$ K L R $\frac{L}{E}$ N I $\frac{G^1}{A^2}$ (Q) P (A) R $\frac{P^2}{A^1}$ D L Y N P (Q) A G						
Glycine max (Glycinin)	G $\frac{V^1}{I^2}$ D E $\frac{N}{T}$ I C T $\frac{L}{M}$ K L R E N I $\frac{G^2}{A^2}$ Q P (S) R $\frac{P^2}{A^1}$ D L Y N P (Q) A G						

Basic subunits were not separated from each other. The superscripts (numbers) give approximate yields, e.g. position 2 ratio of valine to isoleucine is 1/2. It is not always possible to obtain yield data accurately for all positions with two residues. ( ) Indicates some uncertainty in the identification.

EXPERIMENTAL

Seeds of *Vicia faba* L. (var. Triple White) were purchased from the Tyneside Seed Company, Gateshead, Co. Durham, U.K. Commercial soya flour (S200W) was obtained from Central Soya Chicago, U.S.A. Purification of legumin and separation of its subunits was performed as described previously [4]. A reasonably pure glycinin preparation was obtained as the cold-insoluble fraction from a total H_2O extract (incorporating 0.5 mM dithiothreitol and 0.02% (w/v) NaN_3 of soya flour. This was purified further by zonal isoelectric precipitation [4] using modified conditions. The basic subunits of glycinin were prepared by the method used for the separation of legumin subunits, except that the protein was not carboxymethylated and 10 mM 2-mercaptoethanol was included in all buffers. *N*-Terminal amino acid sequences were determined using a Beckman 890c automatic sequencer. Methods were as those described in ref. [8].

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FURTHER CHARACTERIZATION OF PHYTASE FROM *PHASEOLUS AUREUS**

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Key Word Index—*Phaseolus aureus*; Leguminosae; mungbean; phytase; myoinositol phosphates.

Abstract—Phytase purified to homogeneity from germinated mungbean cotyledons was inhibited by EDTA although it did not show any absolute requirement for divalent cations. Sodium fluoride, sodium citrate, mercaptoethanol and *p*CMB also inhibit the phytase activity but L-phenylalanine has no effect on activity. The phytase has a low affinity for inositol monophosphate. The relative rate of dephosphorylation of myo-inositol-1-phosphate and myo-inositol-5-phosphate by phytase is 6 and 18% respectively of that of myo-inositol-hexaphosphate. Mungbean phytase cannot cleave myo-inositol-2-phosphate, 1,2-cyclic inositol phosphate, Na-β-glycerophosphate or *p*-nitrophenylphosphate. The relative rates of hydrolysis of different isomers of inositol hexaphosphate are in the following order: myo-IP_6 > neo-IP_6 > scyllo-IP_6 = D-chiro-IP_6 > L-chiro-IP_6. This enzyme seems to be most active with myo-inositol hexaphosphate.

INTRODUCTION

During the germination of seeds, dephosphorylation of phytate is mediated through phytase (myo-inositol-hexaphosphate phosphohydrolase, EC 3.1.3.8) [1–4]. The mode of action of phytase from other sources had already been partly elucidated [5, 6]. We have purified a phytase from germinating mungbean seeds and shown that the dephosphorylation of myo-inositol-hexaphosphate is sequential [7]. The present paper deals with the further characterization of the enzyme and its specificity towards different substrates and the end product of dephosphorylation reaction.

RESULTS AND DISCUSSION

Effect of divalent and monovalent ions on phytase activity

Activity was increased to *ca* 30–50% above the control

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Abbreviations: *p*CMB, *p*-chloromercuribenzoate; IP, IP_2, IP_3, IP_4, IP_5, IP_6, Inositol mono-, di-, tri-, tetra-, penta-, and hexaphosphate respectively.