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HETEROGENEITY IN SUBUNIT COMPOSITION OF THE LEGUMIN OF *PISUM SATIVUM*

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Key Word Index—*Pisum sativum* L., Leguminosae; pea; legumin; subunit heterogeneity; two dimensional gels.

Abstract—Pea legumin was separated on two dimensional gels into at least 5 acidic (MW *ca* 40000) and 5 basic (MW *ca* 20000) subunits.

INTRODUCTION

Wright and Boulter [1] proposed that the legumin of *Vicia faba* exists as a dimer, each molecule being composed of 3 pairs of subunits linked by disulphide bonds with each pair consisting of 1 acidic (MW *ca* 40000) and one basic (MW *ca* 20000) subunit; a similar structure has been proposed [2, 3] for pea legumin. In view of the subunit heterogeneity of each type of subunit reported in the literature, Derbyshire *et al.* [4] suggested that legumin probably exists in several forms, all with the overall subunit structure given above, but differing in the proportions of the heterogeneous subunits and recently the genetic basis for the heterogeneity of pea legumin subunits has been partially demonstrated [5].

This paper describes the separation of the subunits of pea legumin on two dimensional gels into at least 5 acidic (MW *ca* 40000) and 5 basic (MW *ca* 20000) subunits.

RESULTS AND DISCUSSION

The pattern of the basic subunits on two dimensional gels is given in Fig. 1 and that of the acidic subunits in Fig. 2. Attempts to separate both types of subunit on a single gel of wider pH range (3–10) were unsuccessful. The 5 acidic subunits had the following characteristics: MW 41000, pI 4.9, major; MW 40000, pI 4.75, major; MW 40000, pI 4.6, minor; MW 39000, pI 4.85, major; MW 39000, pI 4.55, minor; there were 3 faint subunit spots and one minor subunit spot (A) whose significance cannot be assessed at this stage. They probably represent minor impurities or partial breakdown products. In addition the 3 spots marked B on Fig. 2 are almost certainly impurities.

The characteristics of the 5 basic subunits were as follows: MW 21500, pI 8.6, major; MW 21500, pI 8.45, minor; MW 21000, pI 8.5, minor; MW 21000, pI 8.7, minor; MW 19000, pI 8.8, minor.

The results obtained using the extract from a single seed were similar to those reported above (data not presented) suggesting that subunit heterogeneity occurred in the cells of an individual.

Although the occurrence of 5 different subunits of both small and large types can be accommodated in the dimer model [1], the fact that additional heterogeneity might

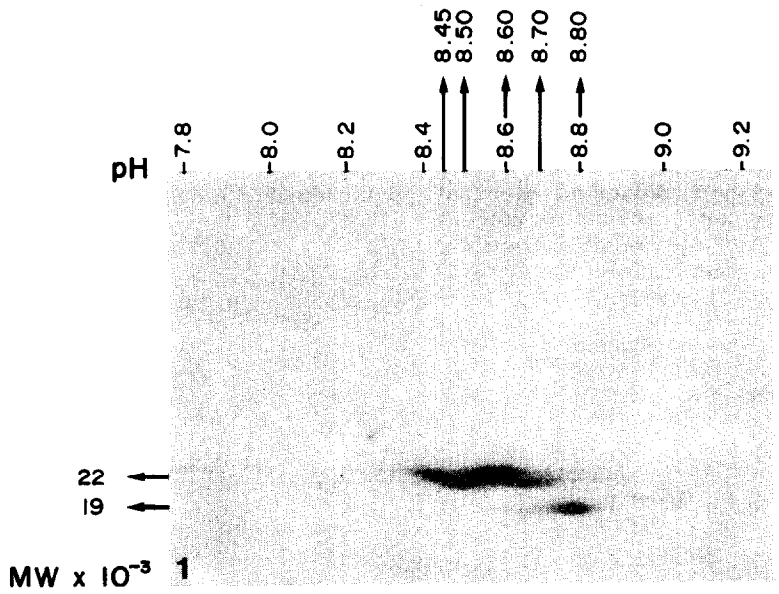

Fig. 1. Two dimensional separation of basic subunits pH range 7–9 (see Experimental). Coomassie blue staining.

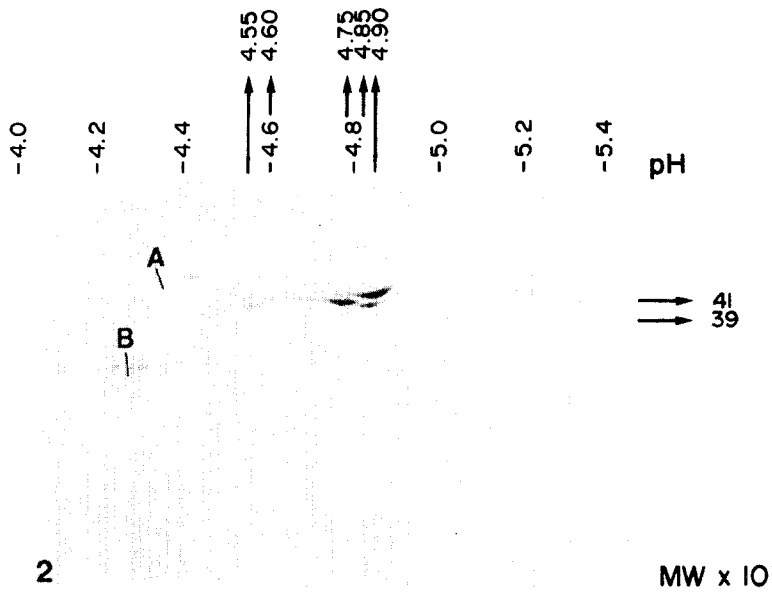

Fig. 2. Two dimensional separation of acidic subunits (see Experimental) pH range 4–6. Coomassie blue staining.

remain undetected, for example conservative amino acid substitutions, makes it likely that there exists more than one form of pea legumin in a cell. These different forms would all be dimers built up of the 3 disulphide-bonded acidic and basic subunit pairs, but the proportions of particular subunits might differ; similar results have been obtained for the legumin of *Vicia faba* (data not presented).

EXPERIMENTAL

Proteins were extracted from seeds of cv Feltham First, ground in 0.05 M borate buffer, pH 8, in a pestle and mortar. Legumin was isolated by zonal isoelectric precipitation [1] and separated by a modification of the 2 dimensional technique of ref. [6], i.e. in the first dimension according to their pIs, by isoelectric focusing in appropriate pH gradients and then the separated subunits were subjected to a second dimension based on their MWs by using SDS gradient pore, slab gel electrophoresis.

Isoelectric focusing. The acrylamide gel concn was 6% and gels contained 6 M urea, and 2% (w/v) ampholines. The anode and cathode electrolytes for focusing the basic subunits were 0.1 M H_3PO_4 and M NaOH, respectively, and the gels were pre-focused for 1 hr at 100 V and the protein subunits focused at 150 V for 16 hr. LKB ampholines consisting of a mixture of pH 7–9 and pH 9–11 were used. The anode and cathode electrolytes for focusing the acidic subunits were 0.1 M H_3PO_4 and 0.1 M NaOH, respectively. LBK ampholines pH 4–6 were used. The gels were pre-focused for 1 hr at 100 V and focused at 200 V for 16 hr using LKB ampholines, pH 4–6.

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NOTE ADDED IN PROOF

Since this paper went to press, an important reference has appeared: Casey, R. (1979) *Biochem. J.* **177**, 509.