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MODIFICATION OF THE BASIC SUBUNITS OF PEA LEGUMIN ON STORAGE

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Key Word Index—*Pisum sativum*; Leguminosae; storage proteins; legumin; modification.

Abstract—Basic subunits of legumin of *Pisum sativum* undergo a modification on storage of dry seeds which increases their apparent MW on SDS–polyacrylamide gel electrophoresis and decreases their pI values.

INTRODUCTION

Legumin, the major storage protein of *Pisum sativum*, undergoes post-translational modification whereby subunit pairs containing disulphide linked acidic and basic subunits are synthesized as a single precursor polypeptide and are subsequently proteolytically cleaved to produce the separate subunits [1]. In addition, Higgins and Spencer [2] have recently described another post-translational modification of pea legumin, which occurs during the final stages of seed development, i.e. during drying out. In this modification, the apparent MW of a basic subunit estimated as 19 000 by SDS-polyacrylamide gel electrophoresis (PAGE) is slightly increased. As these authors state, the reason for this increase in MW is unclear: glycosylation of the subunit is a possibility, but is difficult to reconcile with the absence of significant quantities of carbohydrate in legumin as isolated from mature seeds [3–5]. This paper reports an apparently similar modification of legumin in pea variety Meteor which, however, occurs after seed drying out, i.e. entirely in stored dry seeds.

RESULTS AND DISCUSSION

When total protein extracts of seeds of *P. sativum* cv Meteor were analysed by discontinuous SDS-PAGE a difference in the band pattern in the legumin basic subunits depending on the storage time of seeds was apparent. As shown in Fig. 1, tracks A–F, if mature, dry seeds were analysed immediately after harvesting from the plant the main basic subunits of legumin ran as a single band, MW 22 000 (white arrow) apparently corresponding to the 19 000 MW band of Higgins and Spencer [2]. On storage of the seeds a second band at MW 23 000 (apparently equivalent to the MW 20 000 band of Higgins and Spencer) appeared after 2 weeks, and as the storage time was extended it increased in amount as the band at MW 22 000 decreased in amount (relative to other protein bands). Eventually, after 2 years storage, only the band at MW 23 000 was present (black arrow). Seeds were stored at 4° routinely, but storage at room temperature allowed the same alteration to occur more quickly. Further, this apparent alteration in MW of the legumin basic subunits occurred both on storing whole seeds and on storing finely ground seed meal. Legumin purified from seeds at different storage times showed a band pattern corresponding to that observed in total extracts of pea seeds, as expected (Fig. 1, tracks G–I). From densitometric scanning of stained gels, the shift could be estimated as 50% complete in *ca* 7–8 months at 4°.

The subunits constituting the shifting band undergo a decrease in their isoelectric points during storage, shown by two-dimensional gel electrophoretic analysis employing SDS-PAGE in the first dimension and isoelectric focusing in the second dimension (Fig. 2). The pIs of basic subunit bands of other minor legumin constituents appeared unaltered on storage. The decrease in pI suggested that the shift of the legumin basic subunit band on SDS-PAGE need not actually represent an increase in MW, since we have observed that discontinuous SDS-PAGE systems give separations based on charge as well as size. For instance, maleylation of legumin basic subunits, thus decreasing their positive charge, caused considerable upward shifts in their apparent MW (by *ca* 3000) on discontinuous SDS-PAGE. Further, no changes with storage in the legumin basic subunit band patterns on a continuous SDS-PAGE system were observed, indicating no change in MW. An alteration of net charge on the legumin basic subunits thus seems a reasonable hypothesis. In agreement with this we also observed a slight decrease in mobility of legumin from 2-year-old seeds on non-dissociating PAGE as compared to that from mature seeds stored only for 6 weeks, and an alteration in the elution behaviour of legumin on hydroxylapatite column chromatography with seed storage.

Since this change in the basic subunit 'MW' (i.e. charge) and pI is occurring on storage of the dry seeds, it seems as likely to be due to a non-enzymic chemical alteration of legumin as to a directed enzyme-catalysed process. We would suggest that the most likely alteration in the legumin basic subunit to account for these observations is deamidation of an asparagine or glutamine residue—this will lower the pI of the subunit and decrease its positive charge. This storage alteration in legumin of *P. sativum* indicates that the dry seed is not necessarily chemically inert, and illustrates the care necessary in interpreting band patterns on SDS-PAGE when investigating heterogeneity of proteins, especially in relation to genetic investigations. It seems likely that the storage alteration

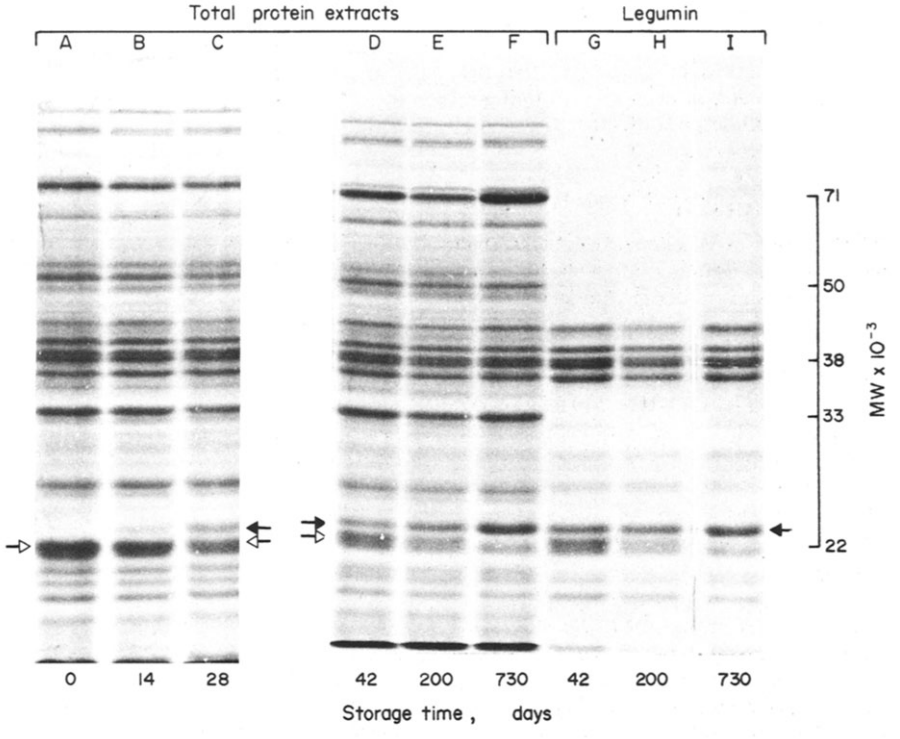

Fig. 1. SDS–PAGE (reducing conditions) of total protein extract (tracks A–F) and legumin (tracks G–I) from seeds of pea variety Meteor stored for various times. White arrow indicates legumin basic subunit band decreasing in amount with storage; black arrow indicates band increasing in amount with storage.

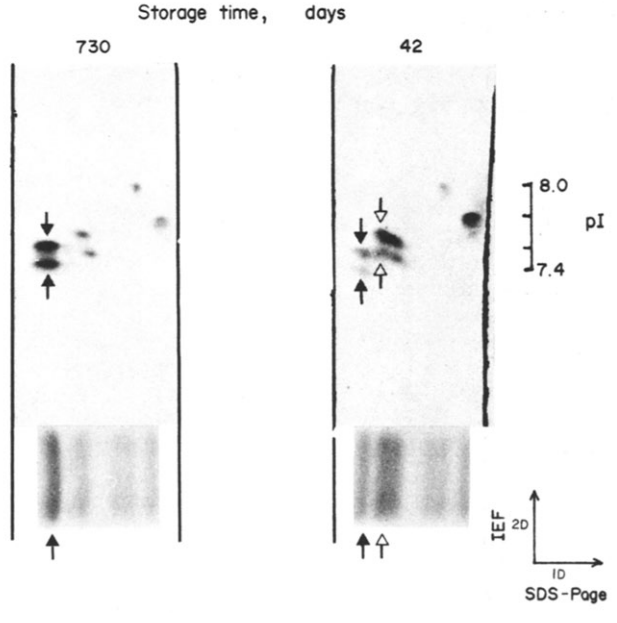

Fig. 2. Two-dimensional gel analysis (SDS–PAGE in first dimension, IEF in second) of legumin basic subunits from seeds of pea variety Meteor stored for various times. White arrows indicate subunits decreasing in amount with storage: black ↑ arrows indicate subunits increasing in amount with storage.

described here is essentially the same as the 'post-translational modification' in legumin described by Higgins and Spencer [2] since we have observed that the rate of alteration differs among pea varieties, and that one variety (cv Feltham First) undergoes the alteration on a time-scale similar to that reported.

EXPERIMENTAL

Mature seeds from *P. sativum* cv Meteor were purchased from Tyneside Seed Stores, Newcastle upon Tyne, U.K. (2-years storage) or were from plants grown in a greenhouse from the same batch of seeds (up to 1 year storage). Legumin was purified from mature seeds by hydroxylapatite column chromatography [5]. Discontinuous SDS-PAGE under reducing conditions was carried out in 12% acrylamide gels by the method of ref. [6]. 2-D gels, SDS-PAGE in the first dimension and isoelectric focusing in 75% formamide in the second were prepared and run as described in ref. [7]. SDS-PAGE in the continuous system was performed according to the method of ref. [8] in a 15% acrylamide gel slab. Non-dissociating PAGE was carried out in 7.5% acrylamide gel slabs using the Laemmli discontinuous system modified as previously described [7].

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