The role of glycosylation in storage-protein synthesis in developing pea seeds

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Abstract. Intact pea (Pisum sativum L.) cotyledons were incubated with \(^{14}C\)glucosamine at several stages of seed development and the resultant radioactively labelled proteins were analysed by gel electrophoresis combined with immunoaffinity chromatography and sucrose gradient fractionation. Glucosamine was incorporated into at least five vicilin polypeptides (approx. molecular weight 70,000; 50,000, two components; 14,000, two components). No incorporation was detected into the subunits of legumin. Tunicamycin at 50 \(\mu\)g/ml largely inhibited glucosamine incorporation but had little effect on the incorporation of \(^{14}C\)-labelled amino acids into cotyledon proteins, including vicilin. The assembly of vicilin polypeptides into full-sized protein oligomers (7-9 S) was also unaffected by tunicamycin. Chromatography on concanavalin A confirmed that glycosylation of cotyledon proteins was inhibited by tunicamycin. It is concluded that glycosylation of most cotyledonary proteins involves lipid-linked sugar intermediates, but that glycosylation itself is not an essential step in the synthesis of vicilin polypeptides nor in their assembly into oligomers.

Key words: Glucosamine – Glycoproteins – Legumin – Pisum (storage protein) – Storage protein, glycosylation – Vicilin.

Introduction

The physicochemical nature of the storage proteins of pea seeds, legumin and vicilin, and their patterns of synthesis during seed development have been extensively studied (Thomson et al. 1978; Casey 1979; Gatehouse et al. 1980; Thomson et al. 1979; Spencer et al. 1980). However, little is known about the mechanisms which control the synthesis of their constituent polypeptides, the assembly of these subunits into protein oligomers and their intracellular transport to, and deposition within, the protein bodies (see Spencer and Higgins, 1979, for discussion). Marshall (1972) has suggested that glycosylation may play an important role in the recognition of some glycoproteins and their transport across membranes. In common with a number of storage proteins of legume seeds, including those from Glycine max (Koshiyama 1966), Phaseolus vulgaris (Racusen and Foote 1971), Vigna radiata (Ericson and Chrispeels 1973) and Lupinus angustifolius (Eaton-Mordas and Moore 1978), the reserve proteins of Pisum sativum have been shown to be glycosylated (Basha and Beevers 1976). Davey and Dudman (1979) have reported that, within the vicilin complex of peas, carbohydrate is mainly associated with the polypeptides of MW, (molecular weight relative to protein standards) 14,000 and 50,000. In the case of pea legumin there is some controversy. In mature seeds, Basha and Beevers (1976) and Davey and Dudman (1979) found appreciable levels of glycosylation of legumin but other workers (Casey 1979; Gatehouse et al. 1980; Hurkman and Beevers 1980) found no attached carbohydrate.

Present evidence strongly indicates that glycosylation of proteins in plants commonly involves the formation of lipid-sugar intermediates (for a recent review, see Elbein 1979), the primary reaction involving N-acetylglucosamine and dolichol phosphate. This reaction is selectively inhibited by the antibiotic tunicamycin in plant (Ericson et al. 1977) as well as in animal tissues (Tkacz and Lampen 1975). The glycosyltransferases which carry out the glycosylation reaction in peas have been shown to be associated with the endoplasmic reticulum (Nagahashi and Beevers 1978).

The aim of the present work was to study the
involvement of glycosylation in the synthesis of individual polypeptides of the storage proteins during pea-seed development and in the assembly of the oligomeric form of these proteins from these individual subunits. Glycosylation of five vicilin polypeptides was detected at various stages in seed development. No glycosylation of legumin polypeptides was observed. In intact cotyledons, inhibition of glycosylation with tunicamycin did not prevent the synthesis of vicilin subunits, nor their assembly into oligomers of characteristic size.

Material and methods
Peas (Pisum sativum L. cv. PI/G 086, a selection from cv. Greenfeast from Rumsey & Son, Sydney, Australia) were grown in artificially-lit cabinets as described previously (Miller and Spencer 1974) but at a temperature of 20°C rather than 25°C. Immature seeds were harvested at intervals during the phase of storage-protein accumulation. Under these growing conditions, the linear phase of protein accumulation extended from approx. 14 to 30 d after flowering. Cotyledons were incubated with radioactive amino acids (2.11 GBq/mmol, 7.4 MBq/ml) were purchased from the Radiochemical Centre, Amersham, U.K. Each cotyledon was incubated with 10–20 μl of these solutions.

Tunicamycin (Eli Lilly & Co., Indianapolis, Ind., USA) was prepared as a stock solution (5 mg/ml) in 10 mM NaOH by heating for 3 min at 37°C. Appropriate aliquots of this were diluted either into distilled water, or into radioactive amino acid or glucosamine solutions for incubating with cotyledons. In all experiments involving tunicamycin, cotyledons were pre-incubated for 2 h with tunicamycin alone and then transferred to radioactive solutions containing tunicamycin for further incubation periods as indicated in Results and Discussion.

Results and discussion
Incorporation of \[^{14}C\]glucosamine into seed proteins. Radioactive glucosamine and amino acids were fed separately to pea cotyledons detached from developing seeds at 14, 20 and 26 d after flowering. These ages cover the most active phase of storage protein synthesis and deposition (Thomson et al. 1979). Incubations were continued for either 4 or 28 h after which extracts were prepared and fractionated by SDS-PAGE. The electrophoretic mobility of the labelled polypeptides were compared with the polypeptides of a protein body extract from mature seeds (Fig. 1, track 1). The latter consists principally of legumin (with polypeptides of MW, 40,000 and 20,000) and vicilin (with polypeptides of MW, 75,000, 50,000, 49,000, 34,000, 30,000, 25,000, 18,000, 14,000, 13,000 and 12,000) (Thomson et al. 1978; Davy and Dudman 1979). In immature seeds the major small subunit of legumin has an MW, of 19,000 rather than 20,000 (Spencer et al. 1980).

Fluorographs of the fractionated extracts (Fig. 1, tracks 2–13) showed that both \[^{14}C\]glucosamine and \[^{14}C\]amino acids were incorporated into polypeptides at all three developmental stages. Qualitative and quantitative differences in the distribution of radioactivity among the polypeptide products were such that the two radioactive substrates generated fairly distinctive labelling patterns. With both glucosamine and amino-acid labelling, the range of products was more diverse after 28 h than after 4 h of incorporation.

Labelling patterns obtained with \[^{14}C\]amino acids have been discussed in detail elsewhere (Spencer et al. 1980). Briefly, the major radioactive polypeptide products were identified as storage protein components and storage protein synthesis was found to account for a major part of total protein synthesis in these cotyledons. The relationship of the glucosamine-labelled products to storage proteins is considered in the next section.

The distinctive qualitative and quantitative differ-