Patterns of protein synthesis during the germination of pea axes, and the effects of an interrupting desiccation period

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Abstract. As germination of axes of Pisum sativum L. seeds progressed, profound quantitative and qualitative changes occurred in the patterns of protein synthesis. This was shown by fluorography of gels following two-dimensional polyacrylamide gel electrophoresis separation of $^{35}$S methionine-labelled proteins. The effects of desiccation during germination on these in-vivo protein-synthesis patterns were followed. Desiccation differentially affected the synthesis of proteins. Usually, however, upon rehydration following desiccation the types of proteins being synthesized were recognizable as those synthesized earlier during imbibition of control, once-imbibed axes: seeds imbibed for 8 h, and then dried, did not recommence synthesis of proteins typical of 8-h-imbibed control seeds, but rather of 4-h-imbibed control seeds. Seeds imbibed for 12 h, and then dried and rehydrated, synthesized proteins typical of 4-h- and 8-h-control seeds. Thus drying of germinating pea axes caused the protein-synthesizing mechanism to revert to producing proteins typical of earlier stages of imbibition. Drying during germination never caused the seed to revert to the metabolic status of the initial mature dry state, however.

Key words: Embryonic axis – Germination (seeds) – Pisum (germination proteins) – Protein synthesis in embryos – Water stress and protein synthesis.

Introduction

Recently there has been a great deal of interest in the effect of different environmental stresses on gene expression in plants, but the effect of water stress on protein-synthesis patterns in plants has received little attention. Mild to moderate osmotic stresses applied to vegetative tissues of higher plants causes some changes in the pattern of protein synthesis (Dhindsa and Cleland 1975; Bewley et al. 1983; Heikkila et al. 1984). Heikkila et al. (1984) also found that water stress led to the appearance of a few novel proteins. Oliver and Bewley (1984) have demonstrated changes in protein synthesis elicited by desiccation of the moss Tortula ruralis; in rehydrated moss, there is a marked synthesis in vivo of new proteins, termed rehydration proteins, the production of which appears to be controlled at the translational level. There is no equally comprehensive account of the effects of drying on protein synthesis during the desiccation-tolerant stage of the germination of seeds.

Desiccation of already-germinated pea seeds (i.e. those from which the radicle has emerged) is lethal if the seeds are dried to below a critically low water content; young seedlings desiccated below this water content lose their capacity to synthesise proteins upon subsequent rehydration. The effect of desiccation during the period until radicle protrusion, however, presents an interesting situation in pea seeds, for in this period the damage induced by desiccation is not lethal, but causes a delay in germination and in in-vivo leucine incorporation upon subsequent rehydration (Lalonde and Bewley 1985).

The work described in this paper was carried out to determine the effect of desiccation on the protein-synthesis pattern in embryonic axes of peas during germination. The two-dimensional polyacrylamide gel electrophoresis technique has allowed us to detect small qualitative and quantitative changes in the pattern of protein synthesis. We present an account of the patterns of protein synthesis occurring during normal germination,
and document the fact that desiccation of germinating pea axes causes the protein-synthesizing mechanism to revert to producing proteins typical of earlier stages of germination.

Material and methods

Plant material. Garden pea seeds (Pisum sativum L. cv. Alaska) were obtained from Burpee Seed Co. (Riverside, Cal., USA). Intact seeds were surface-sterilized with 2% sodium hypochlorite for 5 min, rinsed thoroughly with distilled water, and soaked for 2 h in distilled water. Batches of 10–15 seeds were then allowed to germinate in 9-cm-diameter Petri dishes containing 10 ml distilled water, in darkness at 25°C. When required, embryonic axes were manually excised from the dry pea seeds and germinated in sterile Petri dishes containing sterile distilled water, under similar conditions.

Desiccation treatment. Rapid drying of the seeds was achieved by placing the imbibed seeds, at various stages of germination, over activated silica gel (Tel Tale; Davison Chemical, Baltimore, Md., USA) in glass jars for at least 60 h. Following drying to their original (mature) dry weight, intact seeds or excised embryonic axes were transferred back to moistened filter paper in Petri dishes and allowed to continue germination in darkness as described above.

In-vivo protein labelling. Six axes were placed in 0.3 ml sterile water containing 3.7×10⁵ Bq [³⁵S]methionine (3.7×10¹³ Bq. mmol⁻¹; New England Nuclear, Boston, Mass., USA) for 1.5 h, following 1, 4, 8, 12, 16, 20 or 24 h (re)hydration. After the incubation period, the material was rinsed thoroughly with distilled H₂O, immediately frozen and stored at −70°C until protein extraction.

Extraction of in-vitro labelled proteins for two-dimensional gel electrophoresis. Embryos prelabelled with [³⁵S]methionine were ground in a glass homogenizer in the presence of 2 ml 0.1 M 3-amino-2-(hydroxymethyl)-1,3-propanediol(Tris)-glycine buffer (pH 8.4). The homogenate was cleared by centrifugation at 27000 g for 15 min. Proteins were precipitated with 20% (w/v) trichloroacetic acid (TCA) for 15 min at 4°C. The resultant precipitate was collected by centrifugation, washed with cold 100% ethanol, air-dried, and resuspended in Tris-base and numbered (1–34) for illustrating the changes in the pattern of proteins during the later stages of germination to a pattern characteristic of the late stages (20–24 h). Examination of the fluorographs shows that complex quantitative and qualitative changes in the distribution of proteins synthesized during germination. A comparison of protein synthesis in early-germinating axes (Fig. 1A) with that in the late-germinating axes (Fig. 1B) shows that there is a transition from a pattern which characterizes the early stage (1 h) of germination to a pattern characteristic of the late stages (20–24 h). Examination of the fluorographs shows that complex quantitative and qualitative changes in the distribution of proteins synthesized in vivo take place as imbibition progresses (Fig. 1A–G).

A number of polypeptides have been selected and numbered (1–34) for illustrating the changes in the pattern of proteins synthesized by pea embryonic axes during germination, and in response to desiccation. An examination of the qualitative and quantitative changes of each numbered polypeptide during (re)hydration was carried out. The results are summarized in Table 1. There are selective increases in the synthesis of many polypeptides (e.g. spots 21, 23) and selective decreases in the synthesis of others (e.g. spots 7, 8, 9). But in most cases an initial increase in synthesis is followed by a decrease (e.g. spots 26, 13). A conspicuous change in the pattern of proteins during the later stages of germination is contributed by the synthesis of two new polypeptides (e.g. spots 23, 24). The changes in proteins synthesized during germination (partly shown in Tables 1 and 2) allows their grouping into six germination sets, as follows:

Germination set 1: Proteins unique to the first h of imbibition (spots 1–5).

Results and discussion

Changes in in-vivo protein synthesis during germination. Radicle emergence in a pea seed population starts after 16 h of imbibition, and it takes approx. 23 h for 50% of the seeds to complete germination (Lalonde and Bewley 1985). At various times during germination, newly synthesized polypeptides were labelled with [³⁵S]methionine, isolated, and separated by two-dimensional gel electrophoresis. Fluorographs in Fig. 1 illustrate the changing patterns of polypeptide synthesis in imbibing pea embryonic axes during germination. A comparison of protein synthesis in early-germinating axes (Fig. 1A) with that in the late-germinating axes (Fig. 1B) shows that there is a transition from a pattern which characterizes the early stage (1 h) of germination to a pattern characteristic of the late stages (20–24 h). Examination of the fluorographs shows that complex quantitative and qualitative changes in the distribution of proteins synthesized in vivo take place as imbibition progresses (Fig. 1A–G).

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