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CHANGING PROTEIN SYNTHETIC MACHINERY DURING DEVELOPMENT OF SEEDS OF *VICIA FABA*

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Abstract-Microsomal preparations isolated from seeds at various stages of development have been assayed for amino acid incorporation in vitro, and have also been fractionated on isokinetic sucrose gradients. The changes in protein synthetic activity ***in vivo*** are reflected in the composition and activity of the preparations ***in vitro.***

INTRODUCTION

THERE is a large increase in the amount of rough endoplasmic reticulum just prior to, and during the earlier stages of storage protein synthesis in developing seeds of ***Vicia faba.***[1] The ribosomes of this rough endoplasmic reticulum are synthesized ***de novo,*** and it is not the existing free ribosomes which become attached to newly synthesized membranes at this stage in seed development.[2] This paper reports on the ***in vitro*** amino acid incorporating activity of microsomal preparations, isolated from bean cotyledon cells during various stages of seed development.

RESULTS

The results given in Fig. la show that microsomes from ***30*** day cotyledons are separated on sucrose gradients into a major monosome peak (peak 1), which is preceded by two smaller ribosomal sub-unit peaks. In addition, there are two polysome peaks and a final sharp peak (peak 2) which may be due to much larger polysomes, or to small fragments of endoplasmic reticulum with a few membrane-bound ribosomes attached. The components

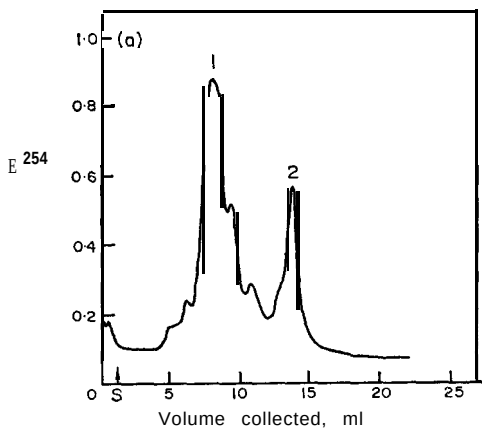

FIG. l(a).

[1] L. G. BRIARTY, D. A. COULT and D. BOULTER, *J. Exptl. Bot. 20, 358 (1969).*

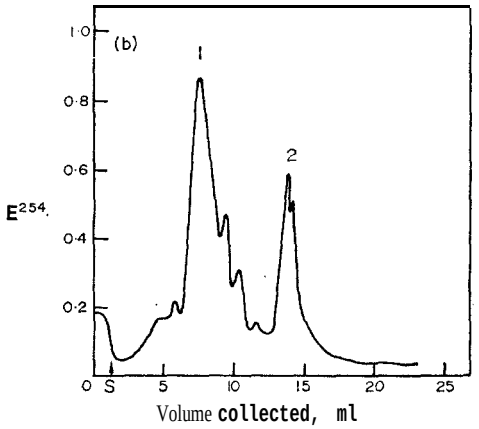

FIG. l(b).

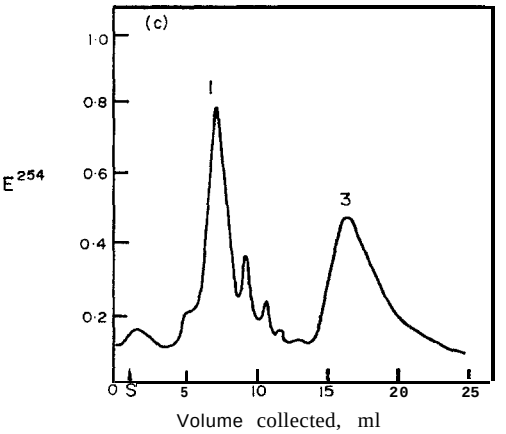

FIG. l(c).

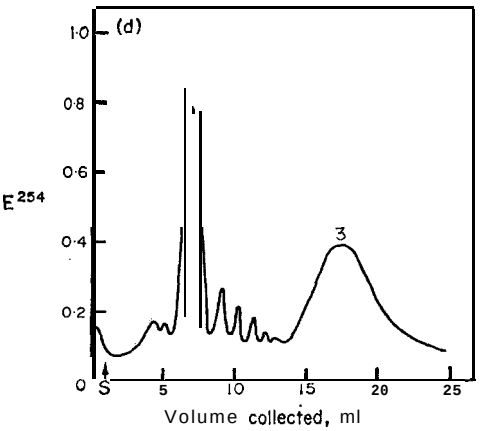

FIG. l(d).

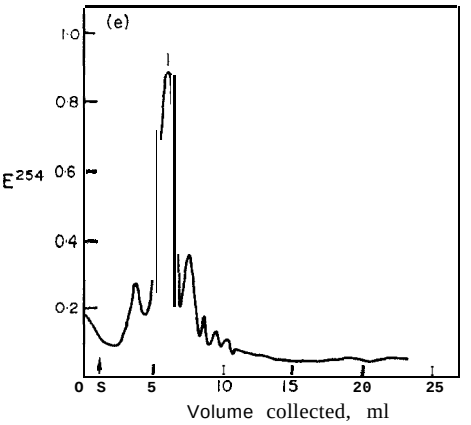

FIG. l(e).

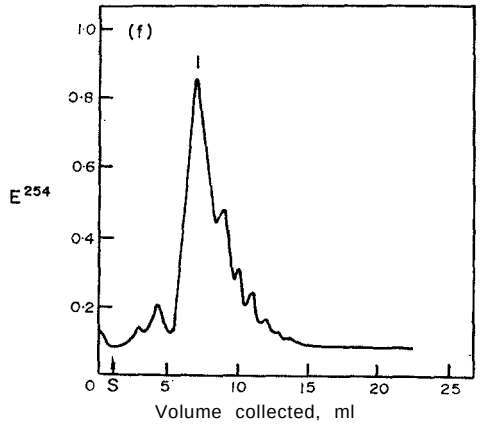

FIG. l(f).

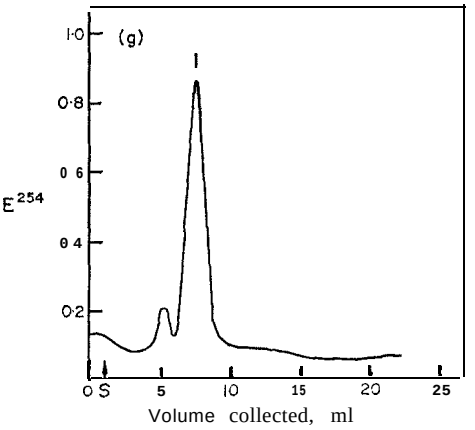

FIG. l(g).

FIG. 1. FRACTIONATION OF MICROSOMAL PREPARATIONS ON SUCROSE GRADIENTS.
(a) 30 days; (b) 40 days; (c) 50 days; (d) 60 days; (e) 70 days; (f) 80 days; (g) 100 days. 15–45% isokinetic gradients were prepared as described previously.[6] O-S = sample layer.

of this peak however, did not migrate sufficiently far through the gradient to correspond to the typical membrane-bound fraction (peak 3) seen in preparations from 50 and 60 day cotyledons (Figs. lc and d). A similar profile to that of the 30 day one was found with material from 40 day cotyledons (Fig. lb), except that three polysome peaks could be distinguished. In preparations from 50 and 60 day cotyledons, there were monosomes (peak 1) and a large broad peak of membrane-bound material (peak 3); 4-5 polysome peaks could be distinguished in the free ribosomal area. No membrane-bound material was present in microsomes prepared from 70 and 80 day cotyledons, although polysomes were still present in the free ribosomal fraction (Figs. 1e and f). In preparations from 100 day seeds, all traces of polysomes were lost, and there was a single large monosome peak (peak 1), with some evidence of sub-units (Fig. lg).

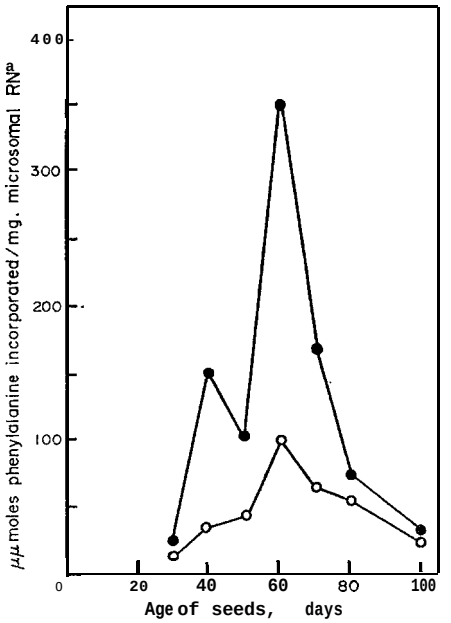

FIG. 2. *In vitro* AMINO ACID INCORPORATING ACTIVITY DURING SEED DEVELOPMENT. ●——● + poly U; ○——○ − poly U.

Figure 2 shows the changes in the *in vitro* amino acid incorporating activity of microsomes extracted from the cotyledons of developing seeds of various ages. In the absence of poly U, incorporation was greatest in preparations from 60 day seeds. In the presence of poly U there were two peaks of phenylalanine incorporation, the first with 40-day-old seeds, the second, which was the greater, with 60-day-old seeds. Material from 30- or 100-day-old seeds had very little activity, both in the presence or absence of poly U.

DISCUSSION

The maturation of *Vicia faba* seeds takes about 100 days and characteristic anatomical and biochemical changes occur, which can be divided into three main phases.' (1) phase of rapid cell division, followed by cell expansion, during which period the major organs of the embryo are formed (approximately l-40 days); (2) a period of intense synthetic activity when the storage proteins and starch accumulate (approximately 40-70 days);

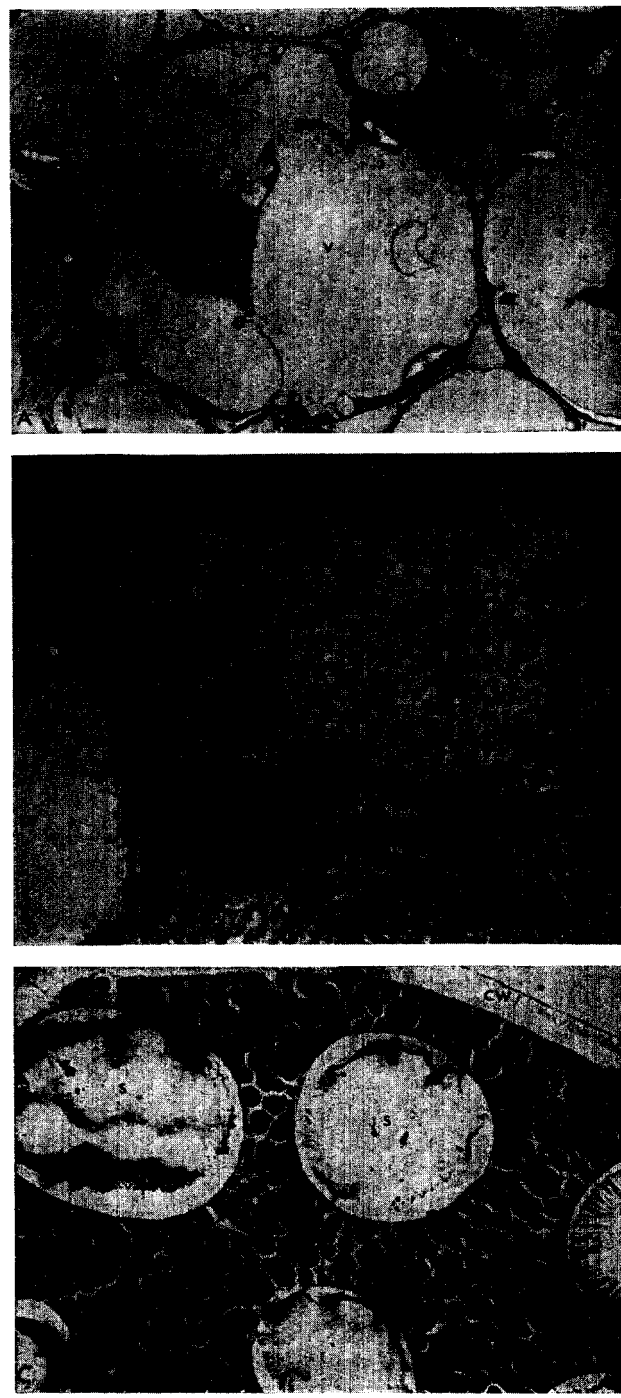

FIG. 3A, B, C. ELECTRON MICROGRAPHS OF *Vicia faba*.

(A) 30 days; (B) 40 days; (C) 70 days. 1 mm slices of cotyledon were fixed in 4% Glutaraldehyde buffered at pH 7, post-fixed in 1% osmium tretoxide, dehydrated in ethanol and embedded in araldite. Silver sections were cut on an LKB ultratome, using glass or diamond knives. The sections were stained in uranyl-acetate and lead citrate prior to examination in an AEI EM6b electron microscope. (Courtesy of A. Cobb and R. Swinhoe.) cw—cell wall; er-endoplasmic reticulum; v—vacuole; pb—protein bodies; n—nucleus; m—mitochondrion; s—starch grain.

(3) after completion of this synthesis there is a final period of maturation and dehydration. Electron microscope studies show that the cotyledon cells from 30-day-old seeds are highly vacuolate with many free ribosomes and no protein bodies (Fig. 3A). After 40 days there is a tremendous proliferation of rough endoplasmic reticulum, and storage protein synthesis leads to traces of protein which can be distinguished as electron-dense areas around the periphery of protein bodies (Fig. 3B). By 70 days the cells are packed with protein bodies completely filled with protein[1,2] (Fig. 3C). Similar patterns of development have been found in other leguminous plants by Bain and Mercer,[3] Opik,[4] and others. There may be a slight variation of a few days in the onset of these phases, depending upon the position of the bean pod on the plant, time at which flowering occurs during the growing season, and environmental conditions prevailing. The electron micrographs show changes in the proportion of free to membrane-bound ribosomes at the onset of protein storage synthesis, and microsomal preparations therefore have been used in studying changes in *in vitro* amino acid incorporation during seed development. Payne and Boulter[2] separated microsomes prepared from different ages of developing seeds into free and membrane-bound material on discontinuous sucrose gradients. They found that in their preparations the ratio of free to membrane-bound ribosomes was initially high, but this ratio fell as the bean seed matured. The ratios obtained, reflected the proportion of free to membrane-bound ribosomes as seen in electron micrographs of the cotyledons. In the present study isokinetic gradients of greater resolving power have been used, which allow the characterization of the polysome peaks. These confirmed the earlier results showing that only in the 50- and 60-day-old preparations, is there a large broad peak of membrane-bound material. The results of Fig. 2 show that changes in protein synthetic activity during seed development *in vivo*[5] are reproduced *in vitro,* when components all from the same age of seed are incubated in the complete system in the absence of poly U, i.e. maximum activity was obtained with 60-day-old preparations. One explanation of their greater activity could be that they contain larger amounts of messenger RNA per milligram of *r*RNA. However, in the presence of poly U, 60-day-old material was again the most active, indicating that ribosomes of this age are potentially more active *per se.* Experiments in which ribosomes of different ages were incubated with standard *t*RNA and enzyme preparations in the presence of poly U, also showed the greater activity at 60 days, which cannot be ascribed therefore, to more active *t*RNA or enzyme preparations at that particular age. A possible reason could be the presence of protein factors which are associated with the ribosomes at this stage in seed development, making them more active.

It is interesting to note that in the results from Fig. 2, in the presence of poly U there is a double peak of activity, the 60-day-old peak we have already discussed, the other one occurred at 40 days and coincided with the burst of protein synthesizing activity, which precedes storage protein synthesis when the proteins of the endoplasmic reticulum are synthesized.

EXPERIMENTAL

Biological materials, chemicals, and the isolation of the various components of the amino acid incorporating system, are as described in Payne et al.[6] The preparations of isokinetic sucrose gradients and the method of radioactive assay are also described there.

[2] P. I. Payne and D. Boulter, *Planta 84,263* (1969).
[3] J. M. Bain and F. V. Mercer, *Aust. J. Biol. Sci.* 19, 49 (1960).
[4] H. Opik, *J. Exptl. Bot.* 19, 64 (1968).
[5] D. Boulter and 0. J. Davis, *New Phytol.* 67,935 (1968).
[6] E. S. Payne, D. Boulter, A. Brownrigg, D. Lonsdale, A. Yarwood and J. N. Yarwood. *Phytochem.* **10,** 2293 (1971).