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PROTECTION OF POLYRIBOSOMES BY ATTACHMENT TO MEMBRANES AND BY DIETHYLPYROCARBONATE DURING THEIR EXTRACTION FROM *VICIA FABA*

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Key Word Index—*Vicia faba*; Leguminosae; polyribosomes; protection with diethylpyrocarbonate; seed, development.

Abstract—It was shown that membrane-bound polyribosomes of developing cotyledons were protected during extraction by their membrane attachment and by the presence of diethylpyrocarbonate.

INTRODUCTION

SEED development in the Papilionaceae (Leguminosae, Lotoideae) is characterized by a series of hormonal,[1] ultrastructural,[2–4] and biochemical changes, including synthesis of food reserves.[5] Both biochemical and ultrastructural studies[2–4,6,7] implicate membrane-bound polyribosomes as the site of synthesis of the major seed protein reserve, legumin.

Several studies have reported that the size distribution of polyribosomes can be improved by the use of diethylpyrocarbonate as a nuclease inhibitor,[8–10] although hitherto these studies have been restricted to non-specialised germinating tissues with a low content of membrane-bound polyribosomes. We have investigated this point using a developing tissue in which the polyribosomes are mainly membrane-bound.[11]

RESULTS

The isolation of polyribosomes directly from either bentonite or diethylpyrocarbonate protected 38 000 *g* detergent-treated supernatants, gave profiles which are illustrated in Figs. 1 and 2, respectively.

The isolation of microsomes with or without the inclusion of bentonite during the initial homogenization, resulted in a polysomal profile similar to that depicted in Fig. 1. However, if microsomes were isolated in the presence of diethylpyrocarbonate, a pronounced protective effect was exerted on polyribosomal structure (Fig. 3). The incubation of diethylpyrocarbonate protected microsomes for 1 hr at 0° did not affect the polyribosomal profile, though incubation with RNase does convert the polysome structures to monosomes.[12]

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DISCUSSION

The nuclease inhibitor, diethylpyrocarbonate, which was first reported to have a protective effect on polyribosomal structure during the isolation of wheat embryo polyribosomes,[8] also improved the yield of polyribosomes from the cotyledons of developing seeds of *Vicia faba*. Polyribosomal structure is best protected by isolating microsomes in the presence of diethylpyrocarbonate and then releasing the polyribosomes by Triton lysis, following the resuspension of the microsomal pellet. The increased yield of larger polyribosomes was due to the protection of their structure, when attached to membranes, against the sheer forces incurred during the resuspension of the microsomal pellet and not only by protection of the polyribosomes from RNase action. However, polyribosomes released from microsomes, which were not protected by diethylpyrocarbonate, have a degraded profile similar to that seen in Fig. 1. In preparations which included diethylpyrocarbonate, there was an increase in the ribosomal subunit content as compared to preparations which included bentonite. This agrees with the previous observations that diethylpyrocarbonate causes, to some extent, the disaggregation of ribosome units.[8–10]

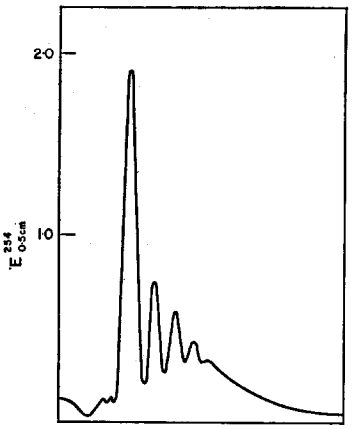

FIG. 1. SUCROSE-GRADIENT SEDIMENTATION PROFILE OF POLYSOMES, FROM 60-DAY-OLD COTYLEDONS, PREPARED FOLLOWING TRITON X100 TREATMENT OF THE 38 000 *g* SUPERNATANT.
The initial homogenization was carried out in the presence of bentonite. Approximately 1 mg of particles were applied to the gradient.

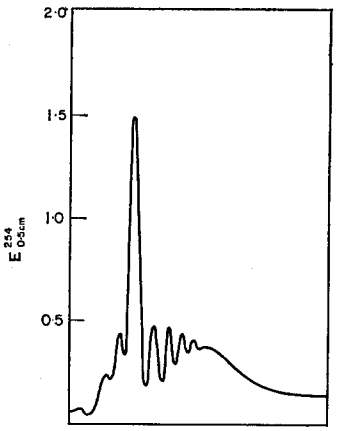

FIG. 2. SUCROSE-GRADIENT SEDIMENTATION PROFILE OF POLYSOMES, FROM 60-DAY-OLD COTYLEDONS, PREPARED FOLLOWING TRITON X-100 TREATMENT OF THE 38 000 *g* SUPERNATANT.
The initial homogenization was carried out in the presence of diethylpyrocarbonate. Approximately 1 mg of particles were applied to the gradient.

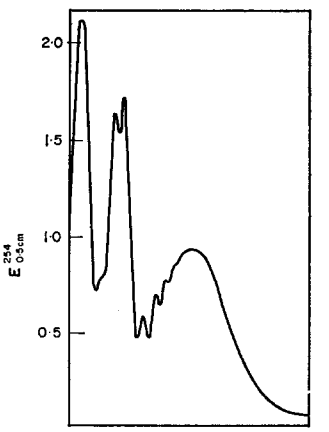

FIG. 3. SUCROSE-GRADIENT SEDIMENTATION PROFILE OF TRITON X-100 DISRUPTED MICROSOMES FROM 60-DAY-OLD COTYLEDONS.
Microsomes were extracted in the presence of DEP and Triton X100 added after resuspension, and the equivalent of 2·0 mg layered onto the gradient.

Large coiled polyribosomes have been demonstrated *in vivo* in the developing cotyledons of both *Phaseolus aureus* and *Vicia faba*.[3,4] As both of these tissues actively synthesise legumin on membrane-bound polysomes, the isolation of intact polyribosomes is a prerequisite for the presumptive identification of legumin messenger RNA. The absence of such large polyribosomes in *V. faba* cell-free extracts, results from the physical problems involved in cell breakage, particle resuspension, particle stability and the RNase content of the tissue.

EXPERIMENTAL

Biological materials. Seeds of *Vicia faba* L., cv. Triple White, were grown in the open. Beans of an average cotyledon wt for a particular age were used.[12]

Chemicals. Chemicals were obtained commercially, except for diethylpyrocarbonate which was a gift from Bayer Chemicals Ltd., Richmond, Surrey.

Isolation of polyribosomes. 10 g tissue was homogenized in a mortar in 3 vol. of 50 mM Tris HCl, 0·5 M sucrose, 16 mM KCl, 5 mM $MgCl_2$, pH 7·5 containing sodium bentonite,[13] to a final concentration of 1 mg/ml. Sodium bentonite was omitted if diethylpyrocarbonate was added. Diethylpyrocarbonate was added to a final concentration of 1% (v/v) and the Tris concentration was adjusted to 0·1 M by the addition of 1/39 vol. 2 M untitrated Tris.[8] The brei was filtered through silk and the filtrate was centrifuged at 38 000 *g* max for 15 min. For the isolation of microsomes the 38 000 *g* supernatant was recentrifuged at 105 000 *g* av. for 90 min. For the isolation of polyribosomes the 38 000 *g* supernatant was made 4% (v/v) with respect to Triton X100 by the addition of 0·4 vol. 10% (v/v) buffered Triton X100 then centrifuged at 105 000 *g* av. for 90 min. The resulting supernatant was decanted and the centrifuge tube walls dried with absorbant paper. The pellets were washed once and then carefully resuspended in a small vol. of 10 mM Tris HCl, 25 mM KCl, 1 mM $MgCl_2$, pH 7·6, using a loose-fitting Teflon homogenizer. Particle concentrations were estimated by the method of Ts'o and Vinograd.[14] Prior to the analysis of microsomes on sucrose gradients, the suspension was made 4% (v/v) with respect to Triton X100 by the addition of 0·4 vol. 10% (v/v) buffered Triton X100. All operations were carried out between 0 and 4°.

Sucrose gradient analysis. 19 ml sucrose gradients were prepared from 10 ml 8% (w/v) sucrose and 19 ml, 40% (w/v) sucrose buffered with 10 mM Tris HCl, 25 mM KCl, 1 mM $MgCl_2$, pH 7·6.[15] Gradients were centrifuged at 65 000 *g* av. for 2 hr in the 3 × 23 ml rotor of the MSE superspeed 65. Gradients were fractionated at 0·5 ml/min with an Isco density gradient fractionator model 180. $E^{254}_{0.5\,cm}$ was measured with an Isco UV analyser model 222 attached to a Servoscribe chart recorder.

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