DEVELOPMENT OF PHOSPHOLIPID SYNTHESIZING ENZYMES IN CASTOR BEAN ENDOSPERM

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1. Introduction

Recent studies have shown that several enzymes responsible for phospholipid synthesis are exlusively located in the endoplasmic reticulum of germinating castor bean endosperm. Phosphatidylcholine [1], phophatidylinositol [2], phosphatidylserine [2] and phosphatidylglycerol [3] are all synthesized by this cellular fraction, and as important structural components of mitochondrial and glyoxysomal membranes [4], establish a role for the endoplasmic reticulum during the biogenesis of these organelles.

The present paper establishes that phosphatidylethanolamine is likewise exclusively made by the endoplasmic reticulum in this tissue.

Further, to examine the possible precursor–product relationship between the endoplasmic reticulum and developing organelles, we have measured the capacity of germinating castor bean endosperm to synthesize phosphatidylcholine and phosphatidylethanolamine during organelle ontogeny.

2. Experimental

Caster bean (*Ricinus communis*) seeds were soaked overnight in running tap water and germinated in the dark in moist vermiculite at 33°C.

Ten endosperm halves, removed from seedlings at various stages of development, were homogenized by chopping for 10 min with a single razor blade in 5 ml of grinding medium contained in a Petri dish on ice. The grinding medium contained 150 mM Tricine (pH 7.5), 10 mM KCl, 1 mM EDTA (pH 7.5), 1 mM $MgCl_2$ and 0.4 M (13% w/w) sucrose. The crude homogenate was filtered through 4 layers of cheese-cloth and adjusted to 10 ml with grinding medium.

A crude pellet containing mitochondria and glyoxysomes was obtained by centrifuging the homogenate at 15 000 *g* and 2°C for 10 min.

To obtain isolated organelle fractions, sucrose density gradient centrifugation was employed. 5 ml of homogenate were layered onto a sucrose gradient which consisted of 25 ml of sucrose solution increasing linearly in concentration from 30 to 60% (w/w) over a 2 ml cushion of 60% sucrose, and topped with a 5 ml layer of 20% (w/w) sucrose. Gradients were contained in 38.5 ml cellulose nitrate tubes. All sucrose solutions contained 1 mM EDTA, pH 7.5. Gradients were centrifuged for 4 hr at 20 000 rev/min in a SW 27 rotor in a Beckman L2 65B ultracentrifuge. After centrifugation, 1.0 ml fractions were collected using a Beckman gradient fractionator.

Fumarase and isocitrate lyase were assayed as described previously [5], using Gilford 222 recording spectrophotometer. Phosphorylcholine-glyceride transferase was assayed as described by Lord et al. [6], except that 1,2-dipalmitin and Tween-20 were omitted from the reaction mixture. 0.1 μCi of CDP [methyl-^{14}C] choline (60 Ci/mole; Radiochemical Centre, Amersham, UK) was used as substrate. Phosphorylethanolamine-glyceride transferase was measured by the same assay using 0.1 μCi CDP [2-^{14}C] ethanolamine (28 Ci/mole; Radiochemical Centre, Amersham, UK).

Radioactive products were counted in Bray's fluid [7] using a Packard Tricarb liquid scintillation counter at an efficiency of 50%.

Protein was determined by the method of Lowry et al. [8] using bovine serum albumin as standard. Sucrose concentrations were determined refractometrically.

3. Results

After sucrose density gradient centrifugation of homogenates prepared from 3 day old castor bean endosperm, several well-defined bands were present on the gradient. The bands with mean buoyant densities of 1.12 g/cm^3, 1.19 g/cm^3 and 1.245 g/cm^3 were identified as endoplasmic reticulum membranes, mitochondria and glyoxysomes from the distribution of the respective marker enzymes phosphorylcholine-glyceride transferase [1], fumarase and isocitrate lyase [5] (fig.1a).

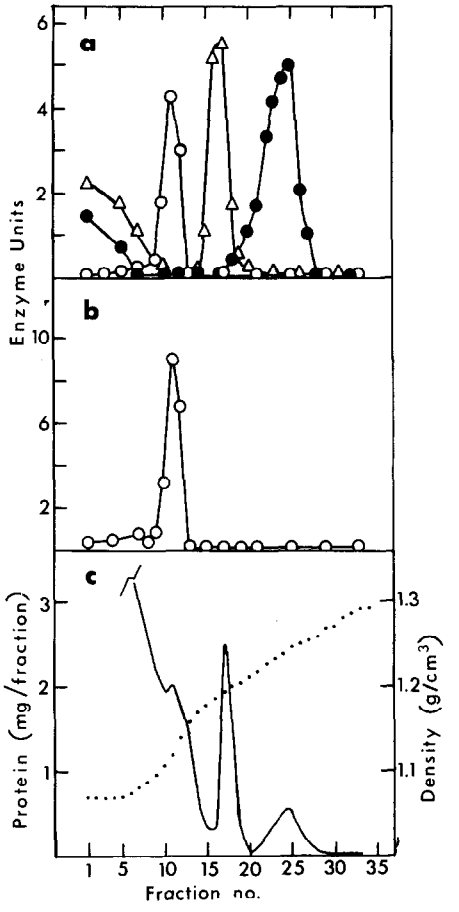

Fig.1. a) Localization of endoplasmic reticulum, mitochondria and glyoxysomes in sucrose gradients using the respective marker enzymes phosphorylcholine-glyceride transferase (o—o—o) nmoles/hr X 20 ; fumarase (△—△—△) μmoles/min X 10; and isocitrate lyase (●—●—●) μmoles/min X 20. b) The position of phosphorylethanolamine-glyceride transferase (nmoles/hr X 10). c) Protein (continuous trace) and sucrose concentration of sequential gradient fractions.

The distribution of phosphorylethanolamine-glyceride transferase across the gradient was identical to that of phosphorylcholine-glyceride transferase (fig.1b), establishing that the endoplasmic reticulum membrane fraction is the exclusive cellular site of synthesis of phosphatidylethanolamine.

The relationship between the capacity of developing endosperm tissue to produce two phospholipids and the synthesis of mitochondria and glyoxysomes is shown in fig.2. The activities of the enzymes catalysing phosphatidylcholine and phosphatidylethanolamine formation increase strikingly during early development, reach a maximum on the third day of germination and subsequently decline rapidly (fig.2a). However, the maximum cellular content of mitochondria and glyoxysomes, as indicated by the recovery of particulate fumarase and isocitrate lyase, occurred on the fourth day of germination (fig.2b). Total protein pres-

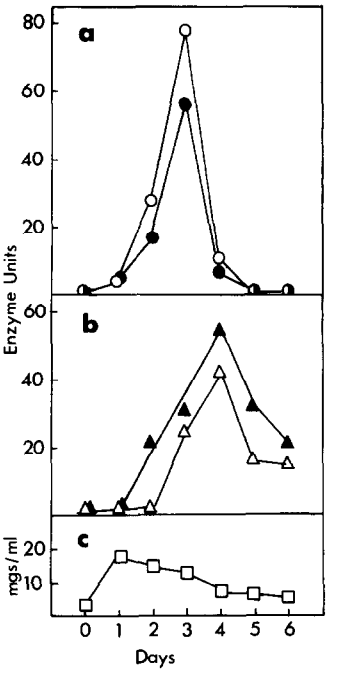

Fig.2. The effect of seedling age on the activities of a phosphorylcholine-glyceride transferase (●—●—●) nmoles/hr X 2 and phosphorylethanolamine-glyceride transferase (o—o—o) nmoles/hr, b) mitochondrial fumarase (△—△—△) μmoles/min and glyoxysomal isocitrate lyase (▲—▲—▲) μmoles/min X2 in the crude organelle pellet and c) total protein present in homogenates from 10 endosperm halves.

ent in the homogenates prepared at various stages of development declined gradually (fig.2c). When expressed on a protein basis, the same activity profile was obtained (data not shown), indicating that the observed changes in enzyme activity recorded during development did not reflect variability in the homogenization step.

4. Discussion

Previous studies have established that the endoplasmic reticulum is the exclusive cellular site of phosphatidylcholine synthesis in castor bean endosperm [1]. The pathways for the incorporation of both choline and ethanolamine into phosphatidylcholine and phosphatidylethanolamine respectively have been shown to involve the same intermediates [9] and, as such, suggest that the final enzymes in the sequence would be located at the same cellular site, i.e. the endoplasmic reticulum. This has been confirmed by the present study.

Phosphatidylcholine and phosphatidylethanolamine constitute approx. 70–80% of the total phospholipid in the glyoxysomal and mitochondrial membranes in castor bean endosperm [4]. Under the present conditions, the cellular content of these organelles reached a maximum after 4 days of germination. It is, therefore, necessary that their membrane constituents, including phospholipids, are available for assembly at or before this time. The data presented in fig.2 show that this requirement is met with respect to phosphatidylcholine and phosphatidylethanolamine in that the maximum rate of synthesis of these phospholipids in the endoplasmic reticulum, is reached on day 3. This may indicate a period of membrane proliferation, perhaps in the endoplasmic reticulum, prior to the utilization of such membranes, or membrane constituents, for organelle assembly.

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