Short Communication

A Comparison of Intrinsic Endoplasmic Reticulum Membrane Proteins in Maturing Seeds and Germinated Seedlings of Castor Bean

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ABSTRACT

The intrinsic membrane proteins of the endoplasmic reticulum from endosperm of maturing and germinated seedlings of castor bean (Ricinus communis) were studied. Preparations were simultaneously subjected to two-dimensional polyacrylamide gel electrophoresis. At least 30 separate proteins were distinguished by staining the gels with Coomassie R-250. The characteristic protein profiles obtained from 0.2 M KCl-washed membranes of each endoplasmic reticulum source are highly reproducible. Of these proteins, three to six that were present in maturing seed were found also in germinating seedlings. In general, the majority of membrane proteins from the endoplasmic reticulum of maturing seed were of a higher molecular weight than those from germinated seedlings.

The protein composition of the ER from germinated seedlings of castor bean has been previously characterized by single-dimensional SDS gel electrophoresis (7, 8, 10) and by isoelectric focusing (9). When used separately, these two techniques yield only a minimal amount of information. However, when combined, unique polypeptides can be resolved individually. The advantage of enhanced resolution offered by two-dimensional gel electrophoresis (16) allowed Vlasuk and Waltz (18) to study the effect of drugs upon ER of rat liver. This technique has not been further exploited in studies of ER from plant sources.

The ER of plants has been implicated in at least two different cellular functions. The synthesis of storage proteins has been shown to take place on ER (3, 5), and the ER is also implicated in the biogenesis of glyoxysomal membranes (for review, see Ref. 4). Thus, a detailed description of the membrane components of the ER would provide information regarding the operation of these distinct functions. This report describes the use of two-dimensional gel electrophoresis as a means to compare the intrinsic membrane proteins of ER from the endosperm of castor bean during separate stages of development; i.e. maturation of the seed and germination of the seedling.

MATERIALS AND METHODS

Tissue Selection. One-hundred-fifty castor beans (Ricinus communis cv. Hale) were germinated in moist vermiculite for 4 days after 24 h of imbibition. Seedlings with mean radicle lengths of 4 cm were selected; the endosperm minus the excised cotyledon were processed as outlined below. One-hundred-twenty maturing castor bean capsules (local variety) were collected (40-60 days after fertilization) and stored at 7°C. The capsules were split transversely, and the endosperm was collected from each seed.

ER Isolation. The endosperm from either the maturing seed or the germinated seedling was homogenized with the aid of an onion chopper in 60 ml of cold grinding medium (11). The homogenate was filtered through Miracloth and centrifuged at 270g for 10 min. Each of six identically constructed linear 20 to 60% (w/w) sucrose gradients (38 ml total volume) containing 100 mM Tricine (pH 7.5), 1 mM EDTA, and 10 mM KCl were loaded with 10 ml of the 270g homogenate and centrifuged at 53,000g for 3 h (14) in a Beckman SW 27 rotor. The ER was removed and stored at -70°C.

Membrane Processing and Gel Electrophoresis. The stored ER in 23 to 25% (w/w) sucrose was allowed to thaw at room temperature; it was then diluted with 2 to 3 volumes of cold 0.2 M KCl, 0.05 M Tricine (pH 7.5) (10). The membranes were collected by centrifugation (100,000g for 1 h), resuspended in Tricine-KCl buffer, and dispersed by means of a tissue homogenizer. The membranes were washed twice in 0.2 M KCl buffer and sedimented by centrifugation (100,000g for 30 min). A sample of this washed ER was assayed for protein by the method of Markwell et al. (15).

Membrane proteins (3 mg) were extracted, using 2% SDS, and separated by isoelectric focusing (2). Proteins were separated in the second dimension by using a linear 10 to 15% SDS polyacrylamide gel, as described by O'Farrell (16). Gels were cast and run (12 mamp each) simultaneously.

Enzyme Assays. Organelles were identified in linear sucrose gradients by measuring respective marker enzymes: NADH Cyt c reductase for ER (14); fumarase for mitochondria (17); and isocitrate lyase for glyoxysomes (12).

RESULTS AND DISCUSSION

ER from both 4-day-germinated and maturing seeds of castor bean was identified in 20 to 60% (w/w) linear sucrose gradients by activity of NADH Cyt c reductase. Contamination of the ER by mitochondrial fumarase in gradients overloaded with homogenized endosperm was 13% and 6%, respectively, for the germinated and maturing organelle (Table I). Further, in maturing castor bean seeds, electron micrographs reveal that golgi membranes constitute only 1% of the total visible membranes relative to ER (J. S. Greenwood, personal communication).

Figure 1 (A and B) represents the integral membrane protein distribution from 0.2 M KCl-washed ER by SDS gel electrophoresis. The membranes were washed with 0.2 M KCl prior to

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extraction with SDS. This procedure reduces contamination by loosely bound peripheral proteins (13) and ribosomes or ribosomal constituents (6). Potential artifacts resulting from possible proteolytic digestion of larger mol wt proteins were eliminated by isolating intact organelles prior to disruption. Since Alpi and Beevers (1) have shown that there is no protease activity in crude extracts of castor bean at alkaline pH, organelles were, as a further precaution, disrupted at pH 7.5. Consequently, the gel protein profiles are very reproducible and reveal up to 30 distinct polypeptides when stained with Coomassie R-250. The two-dimensional profile, thus, represents an accurate description of ER integral membrane proteins.

The ER from maturing seeds (Fig. 1A) is characterized by predominantly high mol wt proteins (45,000–68,000). In contrast, ER from 4-day seedlings (Fig. 1B) is characterized by a profile of low mol wt proteins. These low mol wt proteins are also visible in profiles of ER from 3- and 5-day-germinated seedlings (unpublished results). The absence of low mol wt proteins in maturing ER is confirmed by examination of gels overloaded with 2 to 3 times the normal level of protein. These gels do not show the characteristic low mol wt proteins easily visible in profiles of ER protein from germinating seedlings (data not shown). Thus, the protein profiles presented in Figure 1 suggest separate functional roles for the ER during different stages of development.

### Table 1. Marker Enzyme Activity in Linear Sucrose Gradients

<table>
<thead>
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<th>Enzyme</th>
<th>ER</th>
<th>Mitochondria</th>
<th>Glyoxysomes</th>
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<tr>
<td>NADH-Cyt c reductase</td>
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<td>68</td>
<td>316</td>
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<td>Fumarase</td>
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<thead>
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<th>ER</th>
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<tbody>
<tr>
<td>NADH-Cyt c reductase</td>
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<td>324</td>
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</tr>
<tr>
<td>Fumarase</td>
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<tr>
<td>Isocitrate lyase</td>
<td>56</td>
<td>68</td>
<td>16</td>
</tr>
</tbody>
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LITERATURE CITED

6. **Borge N, W Mok, G Kreibich, DD Sabatini** 1974 Ribosomal-membrane

**Fig. 1.** Two-dimensional gel profiles of intrinsic ER membrane proteins from maturing seeds (A) and intrinsic membrane proteins from 4-day-germinated seedlings. (B). Isoelectric focusing gels (pH 3.5 to 10) were loaded with 330 μg of the corresponding SDS extracted protein, then subjected to SDS gel electrophoresis as outlined in the text. Mol wt markers are BSA (68,000), ovalbumin (45,000), carbonic anhydrase (32,000), and lactoglobulin (18,000).
interaction: *In vitro* binding of ribosomes to microsomal membranes. J Mol Biol 88: 559-580


12. **Hock B, H Bevers** 1966 Development and decline of the glyoxylate-cycle enzymes in watermelon seedlings (Citrullus vulgaris Schrad.). Z. Pflanzenphysiol 55: 405-414


