Similarities in the Polypeptide Composition of Glyoxysomal and Endoplasmic-Reticulum Membranes from Castor-Bean Endosperm

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Microsomal fractions, glyoxysomes and mitochondria were isolated from homogenates of germinating castor-bean (*Ricinus communis*) endosperm by sucrose-density-gradient centrifugation. Washed membrane preparations from these cellular fractions were examined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. At corresponding developmental stages the endoplasmic-reticulum and glyoxysomal membranes were strikingly similar in polypeptide composition, at least 16 polypeptides being present in membranes isolated from 3-day-old tissue. Supplying [35 S]methionine to intact endosperm tissue resulted in the labelling of all membrane polypeptides, the specific radioactivity in the endoplasmic reticulum being greater than for equivalent polypeptides of the glyoxysomal membrane. Washing these membranes with sodium deoxycholate solution extensively solubilized protein components, with the exception of a predominant polypeptide of mol.wt. 55000. Mitochondrial membrane preparations differed from those of the endoplasmic reticulum and glyoxysomes in polypeptide molecular-weight distribution and the [35 S]methionine-labelling pattern. The similarity in polypeptide composition between endoplasmic-reticulum and glyoxysomal membranes is discussed in relation to glyoxysome biogenesis.

The early post-germinative growth of fat-storing seeds is characterized by a rapid synthesis of gluconeogenic enzymes and the organelles, glyoxysomes, which house them (Beever, 1969). Glyoxysomes have morphological and biochemical properties typical of microbodies (Hruban & Recheigl, 1969), namely a protein matrix containing characteristic enzymes bounded by a single limiting membrane.

Although such organelles have been isolated and biochemically characterized from a range of eukaryotic cells (Hruban & Recheigl, 1969; Tolbert, 1971), the site of synthesis of their structural and enzymic components and their mode of assembly within the cell is still unclear. Extensive electron-microscopic studies have failed to provide evidence for ribosomes associated with, or located within, such organelles. The DNA, which can be detected in glyoxysomes isolated from castor-bean endosperm, is known to be contaminating nuclear, mitochondrial or proplastid DNA (Douglass *et al.*, 1973). Thus microbodies apparently depend on biosynthetic activity elsewhere in the cell to provide their structural and enzymic proteins. Studies using castor-bean endosperm tissue have established that the major structural phospholipids of the glyoxysomal membrane, including phosphatidylcholine, are exclusively synthesized on the endoplasmic reticulum

(Lord *et al.*, 1973; Moore *et al.*, 1973; Bowden & Lord, 1975).

Electron micrographs of microbodies *in situ* have frequently shown these cytoplasmic organelles in close proximity to sections of the endoplasmic reticulum, and direct continuity between the endoplasmic reticulum and microbody membrane has been reported (Hruban & Recheigl, 1969; Vigil, 1970; Gruber *et al.*, 1970).

In the light of the above observations, it has been suggested (Hruban & Recheigl, 1969) that microbodies might originate from the endoplasmic reticulum by a process of vesiculation. This model for microbody biogenesis implies that their proteins are synthesized on ribosomes attached to the endoplasmic reticulum. Enzymic proteins destined for the microbody matrix are passed from the ribosomes across the membrane of the endoplasmic reticulum before collecting in dilating cisternae, which ultimately bud off from the endomembrane system as an isolated, membrane-bound organelle. An important feature of this model is that the membrane of the microbody is derived directly from that of the endoplasmic reticulum by a process of membrane flow. If this is the case it seems reasonable to suggest that some structural protein components of the microbody membrane may also occur in the endoplasmic-reticulum membrane.

The polypeptide content of washed glyoxysomal membranes has been characterized (Brown *et al.*, 1974; Bieglmayer & Ruis, 1974). In the present study the polypeptide content of castor-bean endosperm glyoxysomal membranes has been compared with that of the purified endoplasmic-reticulum membranes. Striking similarities in the polypeptide composition of these membrane fractions are reported, suggesting a common cellular origin. These results are discussed in relation to the role of the endoplasmic reticulum during glyoxysome biogenesis.

Materials and Methods

Plant material

Seeds of castor bean (*Ricinus communis*) were soaked overnight in running tap water and were grown in moist vermiculite at 30°C; 3-day-old seedlings were chosen carefully for uniformity. The seedlings were detached at the hypocotyl, the endosperm tissue was removed and the cotyledons were discarded. Cellular membranes were radioactively labelled by incubating the intact endosperm tissue with [³⁵S]methionine. Endosperm halves were placed abaxial surface down on moistened filter paper and 10 μ Ci of L-[³⁵S]methionine (260 Ci/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) was applied to the inner surface of each endosperm half. The covered dishes were incubated at 30°C in darkness for 3 h.

Homogenization

Ten endosperm halves were homogenized by chopping for 10 min with a single razor blade in 6 ml of grinding medium contained in a petri dish on ice. The grinding medium contained: 150 mM-Tricine [N-tris(hydroxymethyl)methylglycine], pH 7.5; 10 mM-KCl; 1 mM-EDTA, pH 7.5; 1 mM-MgCl₂; 0.4 M-sucrose (13%, w/w). The crude homogenate was filtered through four layers of cheese-cloth, the residue was extracted with a further 3 ml of grinding medium, and the volume of the filtered homogenate was adjusted to 10 ml with grinding medium.

Separation of cellular organelles

Portions (5 ml) of crude homogenate were layered on to sucrose gradients, 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 3 h at 20000 rev./min (53000 g_{av}) in a SW27 rotor in a Beckman L2 65B ultracentrifuge. After centri-

fugation, 1.0 ml fractions were collected by using a Beckman gradient fractionator.

³⁵S assays

The radioactivity content of separated organelle fractions from endosperm tissue treated with [³⁵S]methionine was determined by counting samples (50 and 100 μ l) of each gradient fraction collected in 10 ml of Bray's (1960) scintillation fluid. Vials were counted for radioactivity in a Packard Tri-Carb liquid-scintillation counter at an efficiency of 70%.

Isolation of organelle membranes

Gradient fractions that showed peak activities of particular organelle marker enzymes were pooled and mixed with an equivalent volume of 50 mM-Tricine, pH 7.5, containing 0.2 M-KCl to disrupt the organelles osmotically (Huang & Beevers, 1973; Bieglmayer *et al.*, 1973). The diluted endoplasmic reticulum and glyoxysome fractions were incubated for 30 min at 25°C. To ensure complete breakage of mitochondria, the diluted mitochondrial fraction was frozen and allowed to thaw at room temperature (20°C). Membranes were recovered by centrifuging at 100000 g and 0–2°C for 30 min. The supernatants were discarded and the membrane pellets were resuspended in 50 mM-Tricine or, when appropriate, this solution supplemented with 0.5% sodium deoxycholate. After 30 min at 25°C, the washed membranes were re-pelleted by centrifugation.

Separation of membrane proteins

Membrane pellets were incubated overnight (16 h) at 37°C in 200 μ l of 2% (w/v) sodium dodecyl sulphate and 2% (v/v) 2-mercaptoethanol in 20 mM-sodium phosphate, pH 7.2. Membrane proteins were separated by electrophoresis in buffers containing sodium dodecyl sulphate as described by Weber & Osborn (1969) on gels polymerized from 10% (w/v) acrylamide.

The gels were stained for 3 h in Coomassie Blue, destained electrophoretically and scanned in a Gilford gel scanner at 600 nm. The gels were calibrated with standard proteins; the relationship between mobility and log (mol.wt.) was linear between 0.2 and 0.9 relative mobility.

Gels prepared from [³⁵S]methionine-labelled membranes were frozen on powdered solid CO₂ and sliced into 1 mm fractions by using a Mickle gel slicer. Each gel slice was solubilized in 0.2 ml of H₂O₂ (30 vol.) for 2–3 h at 70°C and mixed with 10 ml of Bray's (1960) scintillation fluid. Radioactivity was determined by liquid-scintillation counting at 70% counting efficiency.

Enzyme assays

Cholinephosphotransferase (EC 2.7.8.2), fumarase (EC 4.2.1.2) and isocitrate lyase (EC 4.1.3.1) were assayed as described previously (Cooper & Beevers, 1969; Lord *et al.*, 1973).

Other methods

Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Sucrose concentrations were determined refractometrically.

Results

Separation of cellular organelles

Sucrose-density-gradient centrifugation of 3-day-old castor-bean endosperm homogenates effectively separated the major cellular organelle fractions as shown in Fig. 1(a). Endoplasmic-reticulum membranes, mitochondria and glyoxysomes were recovered at mean buoyant densities of 1.12, 1.19 and 1.24 g/ml, as shown by the peak activities of their respective marker enzymes cholinephosphotransferase, fumarase and isocitrate lyase (Lord *et al.*, 1973; Cooper & Beevers, 1969). After centrifugation these fractions were visible in the gradient as discrete, well separated protein bands (Lord *et al.*, 1972) and the distribution of marker enzymes shows that no significant cross-contamination of fractions had occurred. Suspensions of the major subcellular particles were obtained by pooling gradient fractions: fractions 9–11 for the endoplasmic-reticulum membranes, fractions 16–18 for mitochondria and fractions 24–27 for glyoxysomes. Such preparations were considered to contain highly purified organelles.

Developmental changes in the total protein recovered in the major organelle fractions were measured after sucrose-density-gradient separation. In order to avoid contamination of the organelle fractions (particularly of the endoplasmic-reticulum peak, which is near the top of the gradient) with soluble protein, crude homogenates were first centrifuged at 20000g for 15 min and the supernatants discarded. The distribution of organelle marker enzymes between the 20000g pellet and supernatant established that the pellet contained virtually all the endoplasmic-reticulum membranes, mitochondria and glyoxysomes (results not shown). Further, the recovery of enzyme activity on sucrose gradients showed that gentle resuspension of the 20000g pellet in fresh grinding medium before density-gradient centrifugation did not cause significant organelle breakage.

Total protein recovered in the endoplasmic-reticulum peak reached a maximum after 2 days of germination and subsequently declined, particularly

after the third day, whereas mitochondrial and glyoxysomal protein reached a peak around days 3 and 4 (Fig. 2).

Fig. 1. Sucrose-density-gradient centrifugation of castor-bean endosperm homogenates

An extract of ten endosperm halves from 3-day-old seedlings was applied to a sucrose gradient and centrifuged for 3 h at 53000g and 2°C. (a) Endoplasmic-reticulum membranes, mitochondria and glyoxysomes were located on the gradient by the distribution of their respective marker enzymes: cholinephosphotransferase [●, nmol/h ($\times 20$)]; fumarase [Δ , $\mu\text{mol/min}$ ($\times 10$)]; isocitrate lyase [$\circ$, $\mu\text{mol/min}$ ($\times 20$)]. (b) The distribution of ^{35}S among separated gradient fractions from [^{35}S]methionine-treated endosperm tissue. (c) Protein (—) and sucrose concentration (·····) of sequential gradient fractions.

Fig. 2. Development of organelle protein during germination

Homogenates prepared from ten endosperm halves removed from seedlings at various times during germination were centrifuged at 20000g for 15 min. The pellets were gently resuspended in 5 ml of grinding medium and applied to a sucrose gradient that was centrifuged for 3 h at 53 000g and 2°C. The total protein content of the endoplasmic-reticulum (●), mitochondrial (Δ) and glyoxysomal (○) peaks was determined.

Polypeptide composition of membrane fractions

Initially 3-day-old tissue was examined, in which the cellular organelle content is close to maximal (Fig. 2). Glyoxysomal membranes were obtained by fractionating the organelles into membrane 'ghosts' and soluble proteins by osmotic shock in the presence of KCl (Huang & Beevers, 1973; Bieglmayer *et al.*, 1973). The washed membranes were treated with sodium dodecyl sulphate and dissociated polypeptides were separated by polyacrylamide-gel electrophoresis in buffers containing sodium dodecyl sulphate; 16 major protein bands were visible on the stained gels. The distribution and relative staining intensity of these bands showed the characteristic pattern previously reported for glyoxysomal membranes (Brown *et al.*, 1974; Bieglmayer & Ruis, 1974). A densitometer trace of such a gel is shown in Fig. 3(a). Washing the glyoxysomal membranes with sodium deoxycholate before electrophoresis effectively solubilized the bulk of the protein, with the exception of the major proportion of the predominant polypeptide (band 4; Fig. 3b), as previously observed (Bieglmayer & Ruis, 1974). Endoplasmic-reticulum membranes were washed and examined electrophoretically under the same conditions as the glyoxysomal membranes. These membranes had been freed of attached ribosomes during isolation in gradients containing EDTA (Lord *et al.*, 1973). The stained gels typically obtained for the endoplasmic-reticulum membranes were virtually identical in

Fig. 3. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of glyoxysomal membranes from 3-day-old seedlings

Isolated glyoxysomes were broken by osmotic shock, the membranes were washed in 50 mM-Tricine, pH 7.5 (a), or 50 mM-Tricine, pH 7.5, containing 0.5% sodium deoxycholate (b), and examined electrophoretically. A densitometer trace is shown.

appearance with those obtained with glyoxysomal membranes. A densitometer trace of a stained endoplasmic-reticulum-membrane gel is presented in Fig. 4(a), and shows striking similarity in polypeptide composition and distribution to the glyoxysomal membrane (Fig. 3a). Further, washing the endoplasmic-reticulum membranes with sodium deoxycholate before electrophoresis had the same effect as for glyoxysomal membranes; band 4 remained

Fig. 4. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of endoplasmic-reticulum membranes from 3-day-old seedlings

Isolated microsomal fraction was broken by osmotic shock, the membranes were washed in 50mM-Tricine, pH 7.5 (a), or 50mM-Tricine, pH 7.5, containing 0.5% sodium deoxycholate (b), and examined electrophoretically. A densitometer trace is shown.

largely associated with the membrane, whereas the other polypeptides were extensively solubilized (Fig. 4b). The apparent absence of band 6 from the endoplasmic-reticulum gel scan (Fig. 4a) is misleading. Three bands could be resolved by eye in this area of the gel corresponding to glyoxysomal bands 5, 6 and 7, although the absorbance trace indicates the presence of only two components (5 and 7).

The similarity in polypeptide composition between glyoxysomal and endoplasmic-reticulum membranes was emphasized by running gels of mixed membrane

preparations. Stained bands visible on gels loaded with a mixture of glyoxysomal and endoplasmic-reticulum membranes showed patterns identical with gels loaded with the individual fractions (results not shown).

Although bands 1, 2 and 3 appeared to be major components of washed glyoxysomal membranes (Fig. 3a), they were relatively minor components of the endoplasmic-reticulum membrane preparations (Fig. 4a). Previous work indicated that these three bands in glyoxysomal membrane gels represent monomers of soluble matrix enzymes, whereas the other bands of lower molecular weight were structural components of the microbody membrane (Brown *et al.*, 1974), since it has been found that certain glyoxysomal matrix enzymes remain largely associated with membrane 'ghosts' isolated by techniques similar to those used in the present work (Huang & Beevers, 1973).

Endoplasmic-reticulum and glyoxysomal membrane preparations obtained from 1-day-old endosperm tissue were also examined electrophoretically. Stained gels from such preparations differed from those obtained with 3-day-old tissue in the relative staining intensity of the major polypeptides (Fig. 5). In particular bands 10–13, thought to be monomers of structural protein components of these membranes, were more prominent in 1-day-old preparations than in 2-day-old preparations. In contrast the higher-molecular-weight polypeptides (bands 1–5), which were major components of 3-day-old preparations, particularly in the case of glyoxysomes, were less prominent in 1-day-old preparations. This latter finding is consistent with our previous conclusion that these higher-molecular-weight polypeptides may be monomers of soluble glyoxysomal matrix enzymes (Brown *et al.*, 1974); the cellular content of such enzymes is low in 1-day-old tissue, but is considerably higher in 3-day-old tissue (Gerhardt & Beevers, 1970).

Labelling of membrane proteins *in vivo*

[³⁵S]Methionine supplied to excised castor-bean endosperm halves was readily incorporated into membrane proteins. Preliminary experiments established that the rate of [³⁵S]methionine incorporation into cellular protein varied with the developmental age of the tissue and was maximal after 3 days growth under the conditions used here (Bowden & Lord, 1976). The distribution of ³⁵S among organelle fractions isolated from 3-day-old endosperm tissue which had been incubated for 3 h with [³⁵S]methionine is shown in Fig. 1(b). Three peaks of radioactivity were observed corresponding to areas of the gradient occupied by endoplasmic-reticulum membranes, mitochondria and glyoxysomes, although the endoplasmic-reticulum peak was obscured to some extent

Fig. 5. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of endoplasmic-reticulum and glyoxysomal membranes from 1-day-old seedlings

Isolated organelles were broken by osmotic shock, the membranes were washed in 50mM-Tricine, pH7.5, and examined electrophoretically. Densitometer traces are shown of glyoxysomal membranes (a) and endoplasmic-reticulum membranes (b).

by increasing amounts of soluble radioactivity present in fractions towards the top of the gradient.

[^{35}S]Methionine-labelled endoplasmic-reticulum and glyoxysomal membranes were washed and examined electrophoretically. The distribution of radioactivity across the gels revealed the presence of

discrete peaks that correspond exactly in mobility to the major polypeptides visible on stained gels (Fig. 6).

The total ^{35}S content of the endoplasmic-reticulum membranes was 16000c.p.m., whereas that of the glyoxysomal membrane was 11000c.p.m. In typical preparations the endoplasmic-reticulum fraction contained less than half the membrane protein present in the glyoxysome fraction, indicating that the specific radioactivity in the endoplasmic-reticulum membranes was at least 3 times that in the glyoxysomal membranes on a protein or phospholipid basis.

The relative distribution of radioactivity between corresponding polypeptides of the two membrane fractions, summarized in Table 1, reveals interesting differences. The bands (1, 2, 3 and possibly 5), previously thought to represent contamination of the membranes by residual enzymic protein (Brown *et al.*, 1974), contained more radioactivity in the glyoxysome fractions than in the endoplasmic reticulum. In contrast, the remaining polypeptide bands, thought to be monomers of structural protein components of the membranes, were more heavily labelled in the endoplasmic reticulum (Table 1).

Although the endoplasmic-reticulum and glyoxysomal membranes have virtually identical polypeptide composition, when examined electrophoretically, the polypeptide composition of mitochondrial membrane preparations was expected to differ. In particular the inner mitochondrial membrane contains unique enzymic and respiratory proteins, some of which are encoded for by mitochondrial DNA and synthesized within the organelles on mitochondrial ribosomes. Thus the direct developmental relationship proposed for the endoplasmic reticulum and glyoxysomal membrane does not extend to the inner mitochondrial membrane. Stained gels obtained with washed mitochondrial membranes (inner and outer) were clearly different from the endoplasmic reticulum and glyoxysomal membranes in their polypeptide composition and in the distribution of ^{35}S after prelabelling the tissue with [^{35}S]methionine (Fig. 7).

Discussion

The present comparison of the protein components of washed endoplasmic reticulum and glyoxysomal membranes isolated from castor-bean endosperm has revealed a striking similarity between these membranes. The sodium dodecyl sulphate/polyacrylamide-gel electrophoretic system used separates polypeptides on the basis of their molecular weights, and therefore only establishes that these cellular membranes contain polypeptides of the same monomer size. However, the absorbance traces of the stained gels (Figs. 3a and 4a) show that the distribution and relative intensity of the bands is

Fig. 6. Distribution of radioactivity between polypeptides separated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of washed glyoxysomal and endoplasmic-reticulum membranes

Organelles were isolated from 3-day-old endosperm tissue which had been incubated for 3 h with [^{35}S]methionine, broken by osmotic shock, washed in 50 mM-Tricine, pH 7.5, and examined electrophoretically. The data presented show the count rates measured (corrected for a background rate of 25 c.p.m.) (a) Glyoxysomes; (b) endoplasmic reticulum.

the same for both membrane fractions. The membranes also show the same response to washing with sodium deoxycholate solution. With the exception of the major polypeptide of mol.wt. 55000

(band 4), this detergent extensively solubilized the membrane-bound protein (Figs. 3b and 4b). A similar response to washing with deoxycholate has been reported with rough- and smooth-surfaced micro-

Table 1. *Estimated molecular weights and relative radioactivity of membrane polypeptides separated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis*

Organelles were isolated from 3-day-old endosperm tissue which had been incubated for 3 h with [^{35}S]methionine. Molecular weights were calculated from the average relative mobilities of corresponding bands on gels prepared from glyoxysomal and endoplasmic-reticulum membranes; in no case did such mobilities differ by more than 2%.

Band	$10^{-3} \times$ mol.wt.	Radioactivity (c.p.m.)		a/b
		Glyoxysomes (a)	Endoplasmic reticulum (b)	
1	71	290	240	1.20
2	66	650	260	2.50
3	62	360	270	1.30
4	55	870	1150	0.80
5	49	560	340	1.60
6	45	395	—	—
7	43	295	640	0.46
8	39	320	820	0.39
9	36	840	1440	0.60
10	33	215	—	—
11	30	480	810	0.59
12	26	160	745	0.20
13	22	825	1190	0.70
14	17	100	350	0.29
15	16	95	—	—
16	13	365	1490	0.24

somal membranes from rat liver; a single predominant polypeptide of estimated mol.wt. 52000 resisted solubilization (Hinman & Phillips, 1970).

Endoplasmic-reticulum and glyoxysomal membranes both show developmental changes in the relative intensity of their component polypeptides (compare Figs. 3a and 4a with Fig. 5). Thus membranes isolated from 1-day-old tissue apparently contain a greater proportion of structural protein components, whereas membranes from 3-day-old tissue show increased enzymic protein components. However, the similarity in polypeptide composition between the endoplasmic-reticulum and glyoxysomal membranes remains, regardless of the developmental age of the tissue. It is reasonable to speculate that identical protein components are common to both the endoplasmic-reticulum and glyoxysomal membranes, which suggests a common cellular site of origin of such proteins and a possible developmental relationship between these membranes. Such conclusions are compatible with current concepts about microbody biogenesis, namely that these organelles are formed directly from the endoplasmic reticulum by a process of vesiculation.

The polypeptide components of the glyoxysomal-membrane preparations are thought to include

Fig. 7. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of mitochondrial membranes from 3-day-old seedlings*

Mitochondria isolated from endosperm tissue which had been incubated for 3 h with [^{35}S]methionine were broken by freezing and thawing, washed in 50 mM-Tricine, pH 7.5, and examined electrophoretically. The histogram shows the radioactivity of 1 mm gel slices; a densitometer trace (----), plotted as relative light-absorbance, is also shown.

contaminating matrix-enzyme proteins, which are readily removed by washing with increasing concentrations of KCl solution, and structural proteins tightly bound to the membrane (Brown *et al.*, 1974; Bieglmayer & Ruis, 1974). It is interesting that the endoplasmic-reticulum membranes appear to contain the same contaminating proteins (bands 1, 2, 3 and 5; Figs. 3 and 4) in addition to identical structural components. This conclusion is consistent with the findings of Gonzalez & Beevers (1974), using endosperm tissue germinated for 2 or 3 days, in which rapid glyoxysome biogenesis is occurring (Gerhardt & Beevers, 1970). Enzymic assays on sucrose-density-gradient fractions established that a substantial proportion of the key glyoxysomal enzymes malate synthase, citrate synthase and malate dehydrogenase is localized in the endoplasmic-

reticulum membrane fraction. Other glyoxysomal enzymes, such as catalase and isocitrate lyase, were not detected in the endoplasmic-reticulum fraction (Gonzalez & Beevers, 1974). These data support the hypothesis that glyoxysomal enzymes are temporarily housed in the cisternae of the endoplasmic reticulum before being sequestered in glyoxysomes. Deliberate breakage of isolated glyoxysomes by osmotic shock has shown that whereas certain enzymes such as catalase and isocitrate lyase are largely solubilized, others, including malate synthase, citrate synthase and malate dehydrogenase, remain largely associated with the membrane and are recovered within the resealed glyoxysomal 'ghosts' (Huang & Beevers, 1973; Bieglmayer *et al.*, 1973). The inevitable breakage of the endoplasmic-reticulum structure during homogenization may have differential effects on glyoxysomal enzymes present in the cisternae. Certain enzymes such as catalase and isocitrate lyase might be largely lost, whereas others, for example malate synthase, remain largely trapped in the sealed membrane vesicles which constitute the endoplasmic-reticulum fraction (Lord *et al.*, 1973).

The formation of glyoxysomes from a section of the endomembrane system, which accumulates glyoxysomal enzymes, dilates and is finally pinched off as an isolated microbody, implies that the endoplasmic-reticulum membrane gives rise directly to that of the glyoxysome. Previous studies have shown that the major structural phospholipids of the glyoxysomal membrane are synthesized by enzymes which are exclusively confined to the endoplasmic-reticulum membrane (Lord *et al.*, 1973; Moore *et al.*, 1973; Bowden & Lord, 1975). The similarity in polypeptide composition of these membranes suggests a developmental relationship, which has often been implied before on the basis of the close spatial association of these cellular fractions repeatedly observed in electron micrographs (Hruban & Recheigl, 1969; Vigil, 1970).

Pulse labelling of the tissue with [35 S]methionine resulted in a higher specific radioactivity in polypeptides of the endoplasmic-reticulum membranes than in their glyoxysomal equivalents, consistent with a possible precursor-product relationship (Fig. 6). Thus both phospholipids and structural proteins may be synthesized in the endoplasmic reticulum and then inserted into a developing membrane system, part of which may differentiate into the glyoxysomal membrane. Certain differences in functional protein components of endoplasmic-reticulum and glyoxysomal membranes from castor-bean endosperm have been noted. For instance, the endoplasmic-reticulum membranes actively catalyse phospholipid synthesis, whereas glyoxysomal membranes are inactive (Moore *et al.*, 1973). Major differences

were not found here in the protein patterns obtained with these two membrane fractions (Fig. 3a and 4a). In particular, additional polypeptides, which might be phospholipid-synthesizing enzymes, were not found uniquely associated with the endoplasmic reticulum. It is possible that such enzymes represent only a small proportion of the total membrane protein and that their presence, or absence, is not detected by the analytical procedures used here. Alternatively, the enzymes might exist in the glyoxysomal membrane in the form of inactive apoproteins having lost, during differentiation, the prosthetic groups or activating factors available in the endoplasmic reticulum.

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