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Subcellular Distribution of Gluconeogenetic Enzymes in Germinating Castor Bean Endosperm[1]

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ABSTRACT

The intracellular distribution of enzymes capable of catalyzing the reactions from oxaloacetate to sucrose in germinating castor bean endosperm has been studied by sucrose density gradient centrifugation. One set of glycolytic enzyme activities was detected in the plastids and another in the cytosol. The percentages of their activities in the plastids were less than 10% of total activities except for aldolase and fructose diphosphatase. The activities of several of the enzymes present in the plastids seem to be too low to account for the *in vivo* rate of gluconeogenesis whereas those in the cytosol are quite adequate. Furthermore, phosphoenolypyruvate carboxykinase, sucrose phosphate synthetase, and sucrose synthetase, which catalyze the first and final steps in the conversion of oxaloacetate to sucrose, were found only in the cytosol. It is deduced that in germinating castor bean endosperm the complete conversion of oxaloacetate to sucrose and CO_2 occurs in the cytosol. The plastids contain some enzymes of the pentose phosphate pathway, pyruvate dehydrogenase and fatty acid synthetase in addition to the set of glycolytic enzymes. This suggests that the role of the plastid in the endosperm of germinating castor bean is the production of fatty acids from sugar phosphates, as it is known to be in the endosperm during seed development.

There is a massive conversion from fat to sucrose in the endosperm of young castor bean seedlings. It has been demonstrated (see Fig. 7) that the β-oxidation of fatty acids and the subsequent conversion of acetyl-CoA to succinate occur in glyoxysomes and that succinate is converted to malate or oxaloacetate in the mitochondria (4). However, the intracellular site of the reactions from oxaloacetate to sucrose is still not known. Kobr and Beevers (18) have shown that the concentrations of glycolytic intermediates and coenzymes in castor bean endosperm do not differ strikingly from those in nongluconeogenetic tissues such as pea and buckwheat seedlings. They suggested (17) that gluconeogenesis may occur in a separate intracellular compartment, the plastid, since several glycolytic enzyme activities were found there. In a previous paper we described a method for preparing intact plastids in high yield from protoplasts from castor bean endosperm (24) which allows a quantitative approach to this problem. We report here the intracellular localization of gluconeogenetic and related enzymes using this method, and conclude that the production of sucrose from oxaloacetate occurs in the cytosol.

MATERIALS AND METHODS

Seeds of castor bean (*Ricinus communis* cv. Hale) were soaked in running tap water for 1 day and germinated in moist Vermiculite at 30 C. The methods for preparation of protoplasts from the endosperm of 4-day-old castor bean seedlings and disruption of the protoplasts in 150 mM Tricine-KOH (pH 7.5)-25% sucrose-1 mM EDTA were described previously (24). The ruptured protoplasts (about 10 mg protein in 2 ml) were layered on a gradient composed of: (*a*) a 1-ml cushion of 60% (w/w) sucrose; (*b*) 13 ml sucrose solution increasing linearly from 30 to 60% sucrose; and (*c*) 1 ml 30% sucrose in an 18-ml tube. All sucrose solutions were prepared in 0.1 mM EDTA (pH 7.5). The gradients were centrifuged at 21,000 rpm for 3 h in a Beckman model L2-65B centrifuge in a Spinco SW 27-1 rotor at 4 C, and sequential fractions (0.4 ml) were collected.

Enzyme Assays. All assays except for those of sucrose-P synthetase (37 C), sucrose synthetase (37 C), RuP_2 carboxylase (25 C), and fructose diphosphatase (25 C) were carried out at room temperature (about 23 C). Assays for sucrose-P synthetase and sucrose synthetase were carried out by measuring the incorporation of labeled UDP-glucose[4] into sucrose-P or sucrose at 37 C for 30 min by a modification of Hawker's methods (14). The reaction mixtures contained, in μmol, K-phosphate buffer (pH 7.5), 5.0; EDTA, 2.5; NaF, 1.25; fructose-6-P, 2.5; UDP-[^{14}C]glucose, 0.5 (1 μCi); in a total volume of 75 μl for sucrose-P synthetase, and Tris-HCl (pH 8.0), 5.0; fructose, 2.5; UDP-[^{14}C]glucose, 0.5 (0.1 μCi); in a total volume of 75 μl for sucrose synthetase. The reactions were stopped by heating in boiling water for 2 min. Twenty μl of reaction mixture were applied to a paper chromatogram. The radioactivity incorporated into sucrose was measured by cutting the chromatogram into 1-cm-wide strips which were assayed for ^{14}C in scintillator solution (0.5%, w/v, PPO in toluene) after chromatography in ethyl acetate-pyridine-water (8:2:1, v/v/v) for 20 h. For the sucrose-P synthetase assay the remainder of the reaction mixture (50 μl) was added to 0.1 ml of 50 mM glycine-NaOH buffer (pH 10.6) containing $MgCl_2$ (5 μmol), 2 μl alkaline phosphatase (Sigma type III), and toluene (10 μl). After incubation at 30 C for 15 h, 20-μl samples were chromatographed as above. The differences between the ^{14}C in sucrose before and after phosphatase treatment represent incorporation of ^{14}C into sucrose-P by sucrose-P synthetase. It was proved that the product was sucrose by invertase treatment (data not shown). The pH of the assay mixture is the optimum pH determined for these enzyme activities in crude homogenates. Hexokinase, hexose-P isomerase, glucose-6-P dehydrogenase, 6-P-gluconate dehydrogenase, phosphoglucomutase, UDP-glucose pyrophosphorylase, 3-P-glycerate kinase, P-glycerate mutase, pyruvate kinase, transaldolase, transketolase, phosphofructokinase, enolase, NAD and NADP triose-

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[4] Abbreviations: UDP-glucose: uridine diphosphate glucose; PEP: phosphoenolpyruvate; RuP_2: ribulose 1,5-bisphosphate.

P dehydrogenase were assayed according to Simcox *et al.* (32). Other assays were those described in the literature as follows, catalase (22), fumarase (28), RuP_2 carboxylase (26), PEP carboxylase (8), PEP carboxykinase (8), pyruvate dehydrogenase (29), aldolase (15), fructose diphosphatase (7), NAD malate dehydrogenase (3), triose-P isomerase (23). Coupling enzymes were obtained from Sigma.

RESULTS

In a previous paper we reported methods for preparing intact plastids in high yield from protoplasts from castor bean endosperm. The enzyme profile of the broken protoplast preparations after sucrose density gradient centrifugations is shown in Figure 1. The prominent protein peak coincides with fumarase activity which is the marker enzyme of mitochondria. Less than 5% of the total fumarase activity was detected in the supernatant fractions. Catalase, the marker enzyme of glyoxysomes, is localized in a fraction at peak density 1.25 g/cm^3 (see below). Only 7% of the total catalase activity was found in the supernatant fractions. RuP_2 carboxylase, the marker enzyme of plastids, was clearly present in a fraction with peak density 1.22 g/cm^3 and less than 2% of the total RuP_2 carboxylase activity was detected in the supernatant fractions. These results show that these three organelles are recovered intact in high yield by this method (24). The distribution of the enzymes which are concerned with gluconeogenesis and related processes was examined in such gradients.

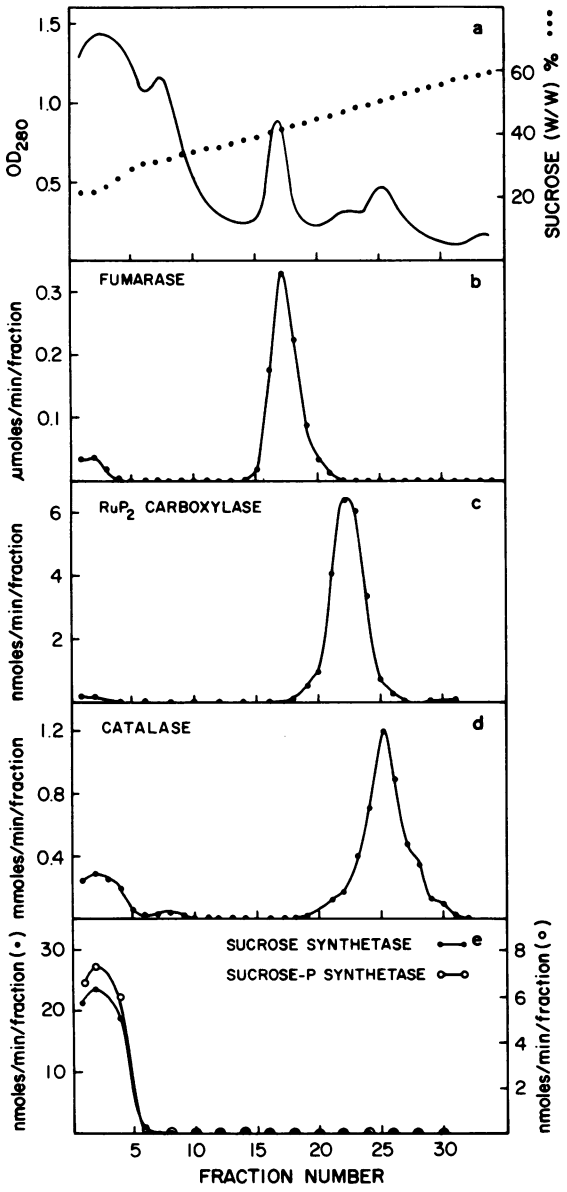

FIG. 1. Distribution of: (a) protein, (b) fumarase, (c) RuP_2 carboxylase, (d) catalase, (e) sucrose synthetase, sucrose-P synthetase in a sucrose gradient of ruptured protoplasts. Sucrose concentrations of sequential 0.4-ml fractions are shown in (a).

Localization of Enzymes Concerned with the Sequence from Oxaloacetate to Sucrose. It has been demonstrated that PEP carboxykinase which catalyzes the reaction from oxaloacetate to PEP and CO_2 (Fig. 7) plays a role in gluconeogenesis in germinating castor bean endosperm (5). This activity is detected only in the supernatant fractions (cytosol) which support previous conclusions (8, 20); no activity is present in the plastid fraction (Fig. 2b). The glycolytic enzymes enolase (Fig. 5b), P-glycerate mutase (Fig. 2a), 3-P-glycerate kinase (Fig. 6c), aldolase (Fig. 4a), fructose diphosphatase (Fig. 4a), hexose-P isomerase (Fig. 3b), phosphoglucomutase (Fig. 3b) as well as phosphofructokinase (Fig. 5b) and pyruvate kinase (Fig. 6b) are clearly present in the plastids as well as in the supernatant fractions. Relatively high amounts of aldolase and fructose diphosphatase are detected in plastid fractions. UDP-Glucose pyrophosphorylase is found in the plastid fractions but there is no peak (Fig. 6c). Sucrose-P synthetase and sucrose synthetase, concerned with sucrose synthesis and breakdown (14), are detected in the supernatant fractions and are not present in the plastid fractions (Fig. 1e). These results show that the cells contain two sets of glycolytic enzymes, one in the plastids and one in the cytosol, but the enzymes catalyzing the first and final steps from oxaloacetate to sucrose are found only in the cytosol.

Localization of Other Related Enzymes. Some enzymes of the pentose cycle, 6-P-gluconate dehydrogenase (Fig. 3a), transaldolase (Fig. 3a), and transketolase (Fig. 5a) are also found in the plastid fractions as reported for the developing castor bean endosperm (32). Most of the pyruvate dehydrogenase activity (Fig. 2a) is found in the mitochondria; the plastid fractions show a significant but small activity and none is detected in supernatant fractions. Glucose-6-P dehydrogenase (Fig. 3a) and NADP-triose-P dehydrogenase (Fig. 4b) known to be present in chloroplasts (25, 30) are not detected in the plastid fractions. NAD malate dehydrogenase (Fig. 6a) activity is found in mitochondrial fractions, glyoxysomal fractions, and supernatant fractions as shown earlier (16) but no clear peak is present in the plastids. Particulate hexokinase activity (Fig. 6b) coincides with the mitochondrial fractions. PEP carboxylase activity (Fig. 2b) is found only in the supernatant fractions as reported in the mesophyll cells of C_4 plants (13). In Tables I and II the contributions of plastid and cytosol to the total enzyme activities are shown; fractions 1 to 8 from the gradient are taken to represent the cytosol activity while fractions 20 to 28 represent the plastids.

DISCUSSION

The isolation of plastids in essentially complete yield from protoplasts has allowed a direct assessment of the amounts of individual enzymes present in these organelles. One difference between the results in this paper and those reported previously (24), which has no bearing on the conclusions, should be noted. In the earlier report, in which castor beans from the 1976 harvest were used for protoplast and organelle preparation, the glyoxysomes were (and are) retained at a density of 1.13 g/cm^3 in the gradients whereas in the conventional preparations from the same endosperm tissue the glyoxysomes appear at their known equilibrium density of 1.25 g/cm^3 (24). In all of the experiments reported here freshly harvested beans from 1977 were used and from these the glyoxysomes obtained from protoplasts or from grinding the endosperm appear in the gradients at a mean density of 1.25 g/

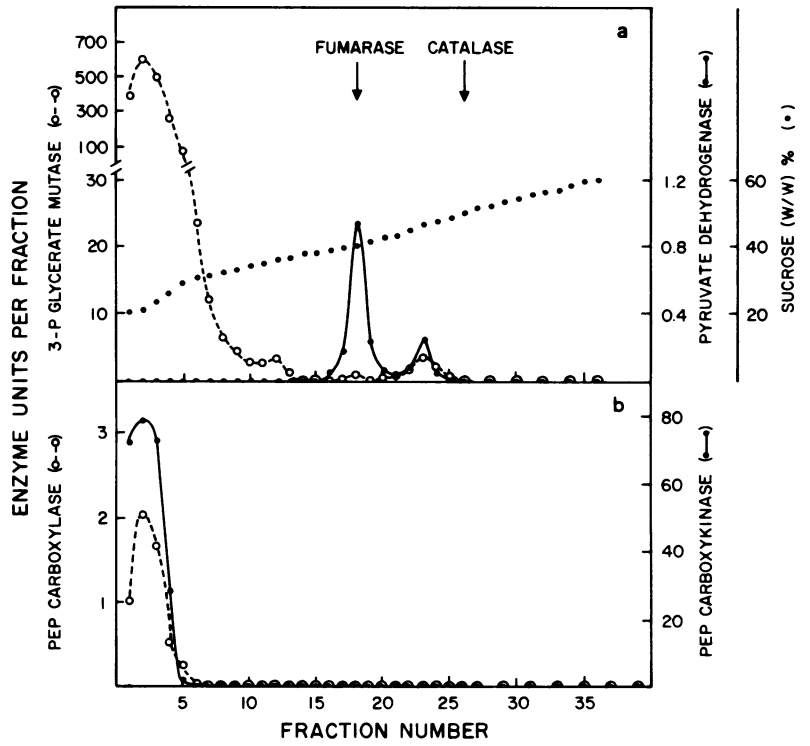

FIG. 2. Distribution of: (a) P-glycerate mutase, pyruvate dehydrogenase, (b) PEP carboxylase, PEP carboxykinase in a sucrose gradient. In this and subsequent figures, arrows show peaks of fumarase and catalase activities in gradient, and one enzyme unit is 1 nmol substrate consumed/min.

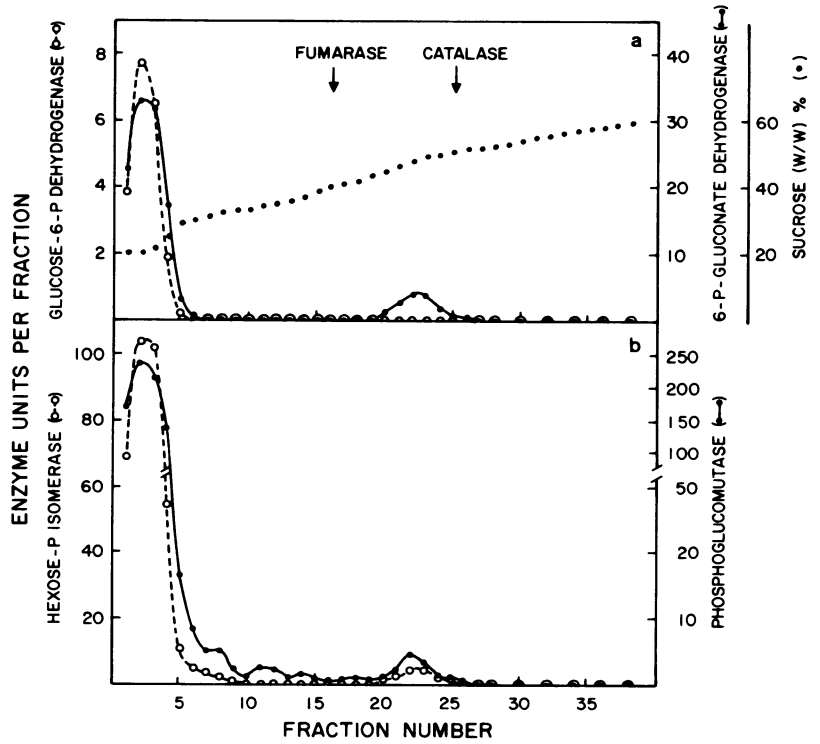

FIG. 3. Distribution of: (a) glucose-6-P dehydrogenase, 6-P-gluconate dehydrogenase, (b) hexose-P isomerase, phosphoglucomutase in a sucrose gradient.

cm^3. We have no explanation for this consistent difference in behavior of the glyoxysomes; the yield of intact plastids and their sedimentation in the sucrose gradient are the same from all of the protoplast preparations that we have used.

In considering the plastid as a possible site of reversed glycolysis (as it is in photosynthesis) the results in Table I show that except for aldolase and fructose diphosphatase, the activities of the glycolytic enzymes in the plastids are all less than 10% of the total (Table I) and some, *e.g.* enolase and P-glycerate mutase, are less than 1%.

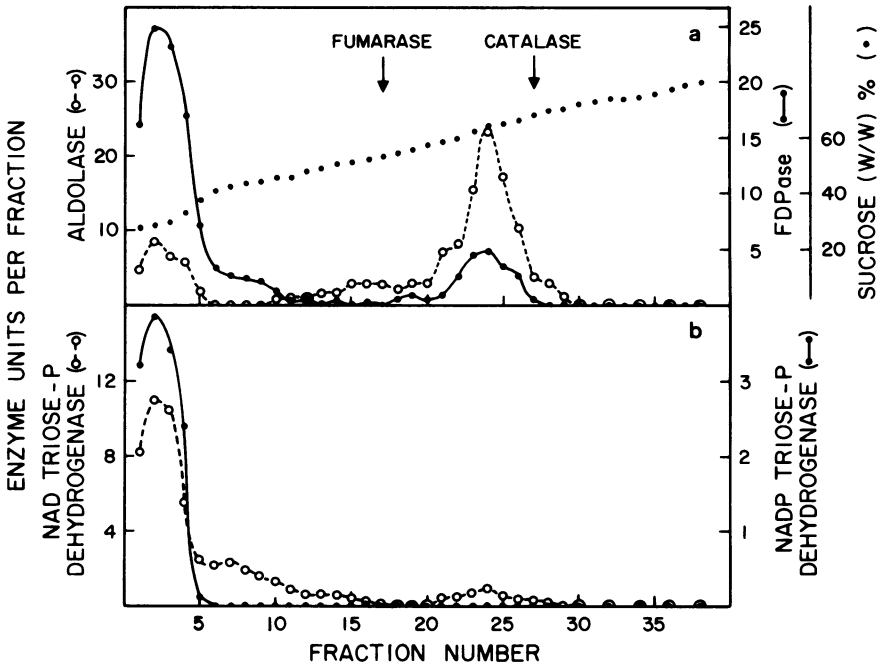

FIG. 4. Distribution of: (a) aldolase, fructose diphosphatase, (b) NAD-triose-P dehydrogenase, NADP-triose-P dehydrogenase in a sucrose gradient.

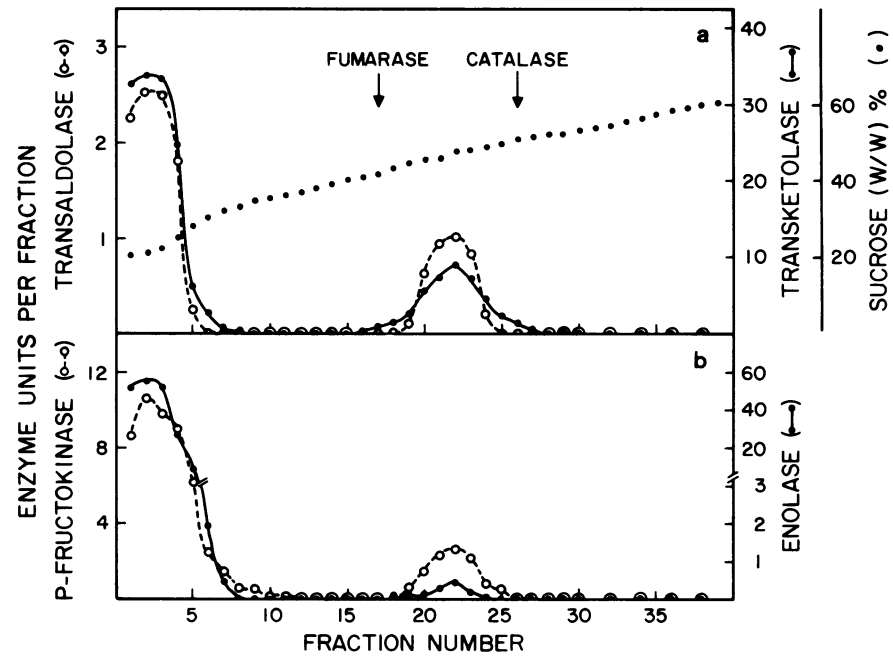

FIG. 5. Distribution of: (a) transaldolase, transketolase, (b) phosphofructokinase, enolase in a sucrose gradient.

To account for the maximum *in vivo* rate of sucrose synthesis, 114 nmol sucrose/min·endosperm at 25 C (9), each enzyme present in the tissue must be capable of handling its substrate at a corresponding rate. The total amount of protein in the endosperm at 4 days is 30 mg. Since the amount of total protein from the protoplasts applied to the gradients was about 10 mg, a minimum capacity of each enzyme corresponding to a rate of sucrose synthesis of 38 nmol/min in the total plastid fraction would be required. It is clear that the cytosolic components of the enzymes are adequate or greatly exceed this requirement (Table I). Within the plastids only the activities of 3-P-glycerate kinase, triose-P-isomerase and aldolase exceed the requirement and those of enolase, P-glycerate mutase, and triose-P dehydrogenase are far below the minimum. On the assumption that the activities measured in *in vitro* assays after gradient separation reflect *in vivo* capacities, we concluded that the maximum flux of carbon through this pathway in the plastids is quite inadequate to account for the rate of sucrose synthesis observed. Thus, reversed glycolysis must occur in the cytosol (Fig. 7). A similar conclusion, based on less extensive data, was reached from work with marrow seedlings (2).

The production of PEP is exclusively cytosolic, and, like chloroplasts (6) the plastids cannot synthesize sucrose from its immediate precursors (Table I). The activity of sucrose synthetase in the cytosol exceeds that of sucrose-P synthetase, as reported

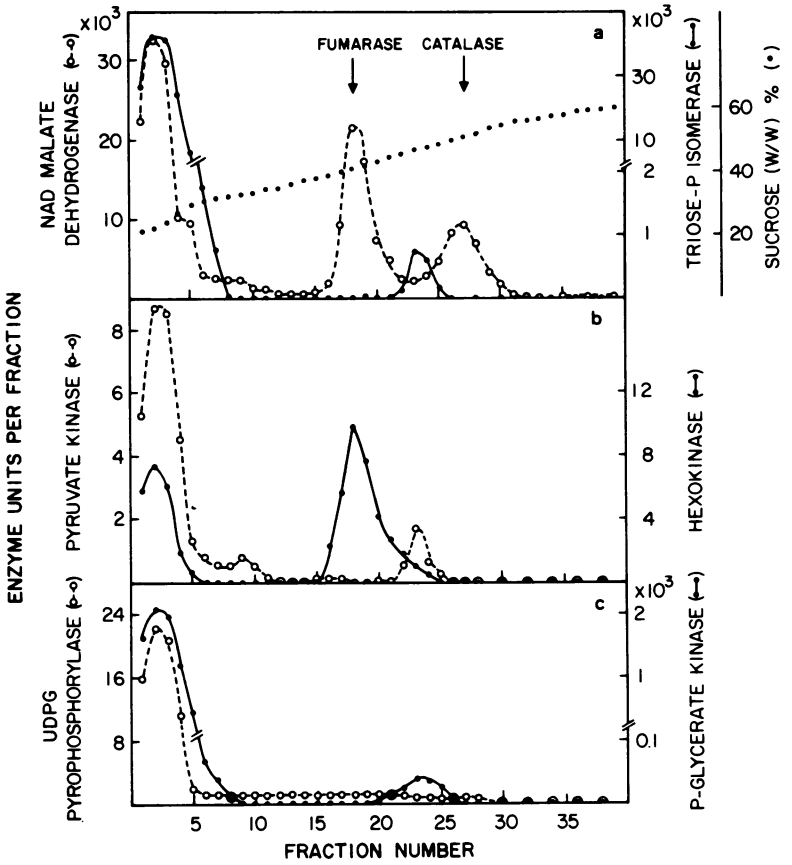

FIG. 6. Distribution of: (a) NAD malate dehydrogenase, triose-P isomerase, (b) pyruvate kinase, hexokinase, (c) UDP-glucose pyrophosphorylase, 3-P-glycerate kinase in a sucrose gradient.

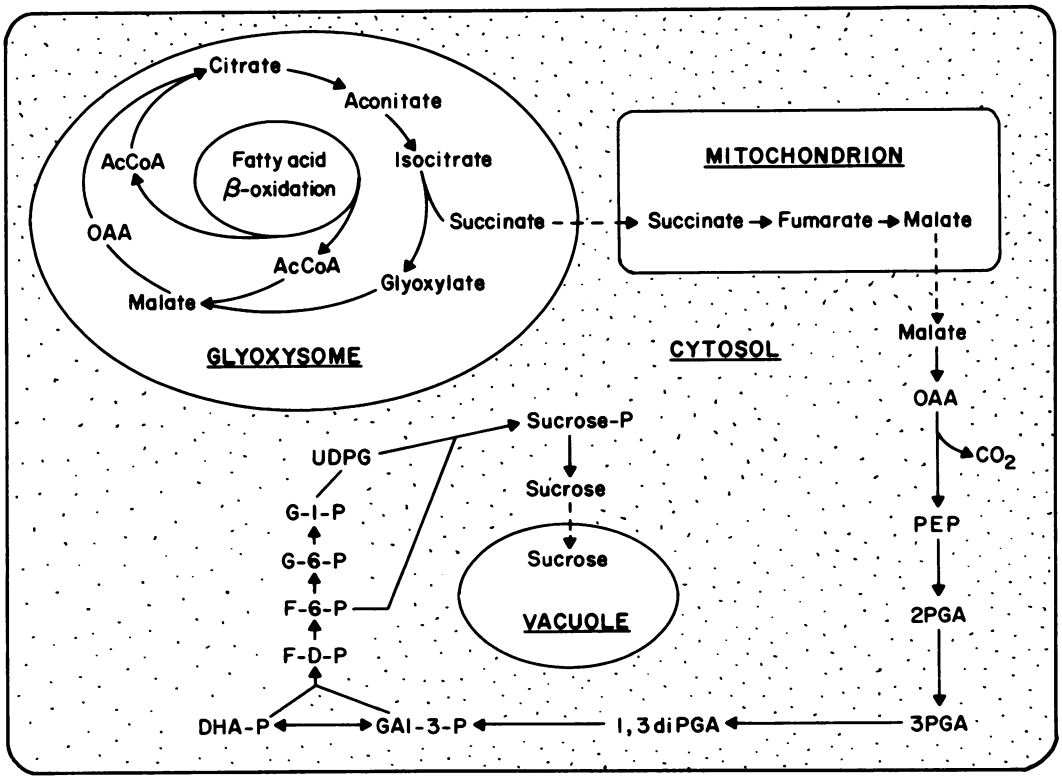

FIG. 7. Intracellular distribution of enzyme systems responsible for conversion of fatty acid to sucrose in castor bean endosperm. In this representation malate leaves the mitochondria and is oxidized in the cytosol, yielding the NADH required for reduction of 1,3-diphosphoglycerate to triose-P. The vacuole is shown as a repository of sucrose (23).

Table I. Gluconeogenetic Enzymes in Plastids and Cytosol.

Values in parentheses show percentages of total activity in the gradient.

	CYTOSOL	PLASTIDS
	nmoles substrate used/min	
PEP carboxykinase	257.5 (99.8)	0 (0)
Enolase	199.8 (99.3)	1.3 (0.6)
P-glycerate mutase	1853.0 (98.8)	9.5 (0.5)
3-P-glycerate kinase	9080.0 (92.7)	364.0 (3.7)
Triose-P dehydrogenase (NAD)	44.8 (85.1)	3.7 (7.0)
Triose-P isomerase	72000.0 (97.7)	1640.0 (2.3)
Aldolase	31.0 (21.8)	104.5 (73.3)
Fructose diphosphatase	97.2 (78.3)	19.7 (15.9)
Phosphoglucomutase	800.6 (96.9)	15.9 (1.9)
Hexose-P isomerase	354.4 (93.9)	18.5 (4.9)
UDP-glucose pyrophosphorylase	76.0 (79.1)	6.4[1] (6.7)
Sucrose-P synthetase	28.4 (100)	0 (0)
Sucrose synthetase	93.5 (100)	0 (0)

[1]No clear peak in plastids.

Table II. Other Enzymes in Plastids and Cytosol.

Values in parentheses show percentages of total activity in the gradient.

	CYTOSOL	PLASTIDS
	nmoles substrate used/min	
Glucose-6-P dehydrogenase	19.8 (100)	0 (0)
6-P-gluconate dehydrogenase	109.1 (87.3)	15.8 (12.7)
Phosphofructokinase	49.3 (80.8)	10.9 (17.9)
Pyruvate kinase	34.1 (84.1)	3.2 (7.9)
Pyruvate dehydrogenase	0 (0)	0.4 (20.0)
RuP_2 carboxylase	0.3 (1.3)	22.1 (97.4)
Transaldolase	9.1 (66.1)	3.7 (26.8)
Transketolase	135.2 (74.7)	40.6 (22.4)
PEP carboxylase	5.7 (100)	0 (0)
Triose-P dehydrogenase (NADP)	13.1 (100)	0 (0)
Hexokinase	15.6 (30.3)	10.2[1] (19.8)

[1]No clear peak in plastids.

previously (14). The major role of the former enzyme appears to be the production of sugar nucleotides, and sucrose-P synthetase with the attendant sucrose-P phosphatase (14) are responsible for sucrose synthesis (12). The observed levels of sucrose-P synthetase (Table I) are barely adequate for *in vivo* sucrose synthesis but these are minimal values. Although the enzyme is activated by Fe^{2+} and K^+ (31) these ions were deliberately omitted from our assays because they also may increase the breakdown of sucrose-P by the phosphatase.

Since the plastids do not appear to be the site of reversed glycolysis, the question of the function of the glycolytic pathway in these organelles arises. In the endosperm tissue during early growth, fatty acids destined for incorporation into phospholipids are synthesized (10); this synthesis occurs in the plastids (33). The fact that the plastids have the capacity to produce acetyl-CoA from sugar phosphates, as well as the NADH and NADPH required for fatty acid synthesis, suggests that these organelles may be the site of the over-all reactions from sugar phosphates to fatty acids as they are known to be in the ripening seed (32, 34). The plastid ATP-citrate lyase recently described (11) offers an additional source of acetyl-CoA for fatty acid synthesis.

In leaves, chloroplasts contain their own set of glycolytic enzymes (*e.g.* 1, 19, 30) including glucose-6-P dehydrogenase and NADP triose-P dehydrogenase (25, 30). Neither of these latter two enzymes is present in the plastids of the endosperm from germinating castor bean, and the glucose-6-P dehydrogenase is not present in the plastids from endosperm in the ripening seedling (32). As in the chloroplasts, several of the plastid enzymes in ripening seeds have been shown to be isoenzymes separable from their cytosolic counterparts (21, 31) and an investigation of the isoenzymes in the germinating castor bean is now proceeding. The recognition that even in nongreen cells the plastids house glycolytic enzymes means that these can no longer be regarded as unique cytosolic markers. It appears justifiable to continue to retain sucrose-P synthetase, sucrose synthetase, PEP carboxylase and PEP carboxykinase in this category.

LITERATURE CITED

1. Anderson LE, JR Advani 1970 Chloroplast and cytoplasmic enzymes. Three distinct isoenzymes associated with the reductive pentose phosphate pathway. Plant Physiol 45: 583-585
2. Ap Rees T, SM Thomas, WA Fuller, B Chapman 1975 Location of gluconeogenesis from phosphoenolypyruvate in cotyledons of *Cucurbita pepo*. Biochim Biophys Acta 385: 145-156

3. ASAHI T, M NISHIMURA 1973 Regulatory function of malate dehydrogenase isoenzymes in the cotyledons of mung bean. J Biochem 73: 217–226
4. BEEVERS H 1975 Organelles from castor bean seedlings: biochemical roles in gluconeogenesis and phospholipid biosynthesis. *In* T Galliard, E Mercer, eds, Fatty Acid and Phospholipid Metabolism in Plants. Academic Press, New York, pp 287–299
5. BENEDICT CR, H BEEVERS 1961 Formation of sucrose from malate in germinating castor beans. I. Conversion of malate to phosphoenolpyruvate. Plant Physiol 36: 540–544
6. BIRD IF, MJ CORNELIUS, AJ KEYS, CP WHITTINGHAM 1974 Intracellular site of sucrose synthesis in leaves. Phytochemistry 13: 59–64
7. BUCHANAN BB 1975 Ferredoxin-activated fructose-1,6-diphosphatase system of spinach chloroplasts. Methods Enzymol 42: 395–405
8. COOPER TG, H BEEVERS 1969 Mitochondria and glyoxysomes from castor bean endosperm. Enzyme constituents and catalytic capacity. J Biol Chem 244: 3507–3513
9. DESVEAUX R, MK KOGANE-CHARLES 1952 Étude sur la germination des quelques graines oléagineuses. Ann Inst Nat Rech Agronom 3: 385–416
10. DONALDSON RP 1976 Membrane lipid metabolism in germinating castor bean endosperm. Plant Physiol 57: 510–515
11. FRITSCH H, H BEEVERS 1979 ATP citrate lyase from germinating castor bean endosperm: localization and some properties. Plant Physiol. In press
12. GANDER JE 1976 Mono- and oligosaccharides. *In* J Bonner, JE Varner, eds, Plant Biochemistry, Academic Press, New York, pp 337–373
13. GUTIERREZ M, SC HUBER, SB KU, R KANAI, GE EDWARDS 1975 Intracellular localization of carbon metabolism in mesophyll cells of C_4 plants. *In* M Avron, ed, Proc Third Intern Congr Photosynthesis. Elsevier, Amsterdam pp. 1219–1230
14. HAWKER JS 1971 Enzymes concerned with sucrose synthesis and transformations in seeds of maize, broad bean and castor bean. Phytochemistry 10: 2313–2322
15. HORECKER BL 1975 Fructose biphosphate aldolase from spinach. Methods Enzymol 42: 234–239
16. HUANG AHC, PD BOWMAN, H BEEVERS 1974 Immunochemical and biochemical studies on isozymes of malate dehydrogenase and citrate synthetase in castor bean glyoxysomes. Plant Physiol 54: 364–368
17. KOBR MJ, H BEEVERS 1968 Distribution of the gluconeogenesis enzymes in the castor bean endosperm. Plant Physiol 43: S-17
18. KOBR MJ, H BEEVERS 1971 Gluconeogenesis in the castor bean enodsperm. I. Changes in glycolytic intermediates. Plant Physiol 47: 48–52
19. LATZKO E, M GIBBS 1968 Distribution and activity of enzymes of the reductive pentose phosphate cycle in spinach leaves and in chloroplasts isolated by different methods. Z Pflanzenphysiol 59: 184–194
20. LEEGOOD RC, T AP REES 1978 Phosphoenolpyruvate carboxykinase and glyconeogenesis in cotyledons of *Cucurbita pepo*. Biochim Biophys Acta 524: 207–218
21. LUCA VD, DT DENNIS 1978 Isoenzymes of pyruvate kinase in proplastids from developing castor bean endosperm. Plant Physiol 61: 1037–1039
22. LÜCK H 1965 Catalase. *In* HU Bergmeyer, ed, Methods of Enzymatic Analysis. Academic Press, New York, pp 885–894
23. NISHIMURA M, H BEEVERS 1978 Hydrolases in vacuoles from castor bean endosperm. Plant Physiol 62: 44–48
24. NISHIMURA M, H BEEVERS 1978 Isolation of intact plastids from protoplasts from castor bean endosperm. Plant Physiol 62: 40–43
25. NISHIMURA M, D GRAHAM, T AKAZAWA 1976 Isolation of intact chloroplasts and other cell organelles from spinach leaf protoplasts. Plant Physiol 58: 309–314
26. NISHIMURA M, T TAKABE, T SUGIYAMA, T AKAZAWA 1973 Structure and function of chloroplast proteins. XIX. Dissociation of spinach leaf ribulose-1, 5-diphosphate carboxylase by *p*-mercuribenzoate. J Biochem 74: 945–954
27. NOMURA T, T AKAZAWA 1974 Enzymic mechanisms of starch breakdown in germinating rice seeds. V. Sucrose phosphate synthetase in the scutellum. Plant Cell Physiol 15: 477–483
28. RACKER E 1950 Spectrophotometric measurements of enzymatic formation of fumaric acid and *cis*-aconitic acids. Biochim Biophys Acta 4: 211–214
29. REID EE, P THOMPSON, CR LYTTLE, DT DENNIS 1977 Pyruvate dehydrogenase complex from higher plant mitochondria and proplastids. Plant Physiol 59: 842–848
30. SCHNARRENBERGER C, A OESER, NE TOLBERT 1973 Two isoenzymes each of glucose 6-P dehydrogenase and 6-P gluconate dehydrogenase in spinach leaves. Arch Biochem Biophys 154: 438–448
31. SIMCOX PD, DT DENNIS 1978 Isoenzymes of the glycolytic and pentose phosphate pathways in proplastids from the developing endosperm of *Ricinus communis* L. Plant Physiol 61: 871–877
32. SIMCOX PD, EE REID, DT CANVIN, DT DENNIS 1977 Enzymes of the glycolytic and pentose phosphate pathways in proplastids from the developing endosperm of *Ricinus communis* L. Plant Physiol 59: 1128–1132
33. VICK B, H BEEVERS 1978 Fatty acid synthesis in endosperm of young castor bean seedlings. Plant Physiol 62: 173–178
34. YAMADA M, Q USAMI 1975 Long chain fatty acid synthesis in developing castor bean seeds. IV. The synthetic system in proplastids. Plant Cell Physiol 16: 879–884