

# The Utilization of Glycolytic Intermediates as Precursors for Fatty Acid Biosynthesis by Pea Root Plastids<sup>1</sup>

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Radiolabeled pyruvate, glucose, glucose-6-phosphate, acetate, and malate are all variously utilized for fatty acid and glycerolipid biosynthesis by isolated pea (*Pisum sativum* L.) root plastids. At the highest concentrations tested (3–5 mM), the rates of incorporation of these precursors into fatty acids were 183, 154, 125, 99, and 57 nmol h<sup>-1</sup> mg<sup>-1</sup> protein, respectively. In all cases, cold pyruvate consistently caused the greatest reduction, whereas cold acetate consistently caused the least reduction, in the amounts of each of the other radioactive precursors utilized for fatty acid biosynthesis. Acetate incorporation into fatty acids was approximately 55% dependent on exogenously supplied reduced nucleotides (NADH and NADPH), whereas the utilization of the remaining precursors was only approximately 10 and 20% dependent on added NAD(P)H. In contrast, the utilization of all precursors was greatly dependent (85–95%) on exogenously supplied ATP. Palmitate, stearate, and oleate were the only fatty acids synthesized from radioactive precursors. Higher concentrations of each precursor caused increased proportions of oleate and decreased proportions of palmitate synthesized. Radioactive fatty acids from all precursors were incorporated into glycerolipids. The data presented indicate that the entire pathway from glucose, including glycolysis, to fatty acids and glycerolipids is operating in pea root plastids. This pathway can supply both carbon and reduced nucleotides required for fatty acid biosynthesis but only a small portion of the ATP required.

Plastids, including chloroplasts and chromoplasts, oil-seed plastids, and root plastids, are the site of de novo fatty acid biosynthesis in plants. This subject has been thoroughly reviewed in a number of works (Stumpf, 1984; Harwood, 1988; Dennis, 1989; Sparace and Kleppinger-Sparace, 1993). Traditionally, most in vitro studies of fatty acid biosynthesis utilize acetate as a radioactive tracer largely because it is efficiently incorporated into fatty acids. This has been justified by the belief that extraplastidic acetate originates through the action of mitochondrial pyruvate dehydrogenase and acetyl-CoA hydrolase (Murphy and Stumpf, 1981), as well as by the occurrence of envelope-bound acetyl-CoA synthetase (Kuhn et al., 1981). However, more recent studies indicate that most plastids contain their own pyruvate dehydrogenase (Reid et al., 1977; Camp and Randall, 1985; Journet and Douce, 1985; Liedvogel and Bauerle, 1986; Kang and Rawsthorne, 1994)

and can readily utilize pyruvate for fatty acid biosynthesis (Yamada and Nakamura, 1975; Smith et al., 1992; Kang and Rawsthorne, 1994). These findings suggest that pyruvate is a physiologically more relevant precursor for fatty acid biosynthesis than free acetate. Furthermore, a number of related studies indicate that nonphotosynthetic plastids contain many of their own glycolytic enzymes with sufficient activities to support observed rates of fatty acid biosynthesis (Yamada and Usami, 1975; Simcox et al., 1977; Miernyk and Dennis, 1983; Journet and Douce, 1985; Denyer and Smith, 1988; Kang and Rawsthorne, 1994). However, these and other similar studies often emphasize that one or more enzymes of the glycolytic pathway are absent or have very low activities, such that carbon flow through this pathway in nonphotosynthetic plastids is restricted (Frehner et al., 1990; Borchert et al., 1993; Trimming and Emes, 1993). Thus, it is assumed that these plastids must partly rely on cytosolic carbon metabolism for the plastidic conversion of hexoses to pyruvate via their own glycolytic pathway.

Studies of the role of plastidic glycolytic metabolism in fatty acid biosynthesis in nonphotosynthetic plastids have emphasized the contribution of this pathway to the production of the ATP, and to a lesser extent, the NAD(P)H required for fatty acid biosynthesis (Kleinig and Liedvogel, 1980; Boyle et al., 1990; Kleppinger-Sparace et al., 1992; Qi et al., 1994). Relatively few studies, however, have described the complete flow of hexose carbon through the glycolytic pathway into fatty acid and glycerolipid biosynthesis in these plastids. Several earlier studies suggested that free Glc or Fru may be utilized as carbon donors for fatty acid synthesis in castor bean leukoplasts (Yamada and Usami, 1975; Miernyk and Dennis, 1983; Dennis et al., 1985). However, fatty acid biosynthesis from these latter precursors was much less active and not well characterized. Recently, Kang and Rawsthorne (1994) demonstrated that Glc-6-P was utilized for fatty acid biosynthesis by isolated plastids from developing rapeseed embryos. In a related study, Smith et al. (1992) showed that malate, via NADPH malic enzyme and pyruvate dehydrogenase, was an extremely effective precursor for fatty acid synthesis in castor bean leukoplasts.

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Abbreviations: MAG, DAG, TAG, mono-, di-, and triacylglycerol, respectively; PA, phosphatidic acid; PC, phosphatidylcholine; PG, phosphatidylglycerol; PGA, phosphoglycerate; PI, phosphatidyl inositol.

Studies of pea (*Pisum sativum* L.) root plastids from several laboratories (including ours) are not in complete agreement as to whether or not an uninterrupted glycolytic pathway from Glc to pyruvate operates within these plastids. Borchert et al. (1993) and Trimming and Emes (1993) both have independently measured the individual activities of glycolytic enzymes in pea root plastid preparations. They have indicated that complete glycolytic carbon flow in these plastids may be prevented, since the activities of hexokinase and PGA mutase were very low or essentially absent. Their evidence further suggests that pea root plastids can only metabolize hexose phosphates as far as the formation of 3-PGA. 3-PGA must then be transported out of the plastid, where cytosolic phosphoglyceromutase converts it to 2-PGA, which is then transported back into the plastid to form pyruvate. In contrast, other workers (Bowsher et al., 1992) have shown that pea root plastids evolved  $^{14}\text{CO}_2$  in the presence of  $[3,4\text{-}^{14}\text{C}]\text{Glc}$ , suggesting that the complete pathway from Glc to acetyl-CoA may be present in these plastids. Similarly, our previous results with pea root plastids (Qi et al., 1994) have shown that unlabeled hexoses and trioses can provide some of the energy required for fatty acid biosynthesis and that glycolytic metabolism in these plastids might also provide carbon to support fatty acid biosynthesis. To help clarify the glycolytic capacity of pea root plastids and evaluate its role in providing carbon for fatty acid synthesis, we examined the capacity of these plastids to utilize  $[\text{U-}^{14}\text{C}]\text{Glc}$  and  $[\text{U-}^{14}\text{C}]\text{Glc-6-P}$  for fatty acid and glycerolipid biosynthesis in comparison to other known precursors for fatty acid biosynthesis. We report here our findings in these studies.

## MATERIALS AND METHODS

### Plant Material and Isolation of Plastids

Seeds of pea (*Pisum sativum* L. cv Improved Laxton's progress) were germinated under sterile conditions (Sparace et al., 1988). Plastids isolated from germinating pea roots (Kleppinger-Sparace et al., 1992) were purified by centrifugation through 10% Percoll (Trimming and Emes, 1993) and washed and resuspended without BSA in 1.0 mM bis-tris-propane (pH 7.9) containing 0.33 M sorbitol to give approximately  $1.0 \text{ mg mL}^{-1}$  plastid protein.

### Isotope Incorporation Studies

$[1\text{-}^{14}\text{C}]\text{Acetate}$ ,  $[\text{U-}^{14}\text{C}]\text{Glc-6-P}$ , and  $[\text{U-}^{14}\text{C}]\text{Glc}$  were obtained from ICN Biomedicals, Canada (Mississauga, Ontario).  $[2\text{-}^{14}\text{C}]\text{Pyruvate}$  was from Dupont Canada (NEN Research Products, Mississauga, Ontario) and  $[\text{U-}^{14}\text{C}]\text{malate}$  was from Amersham Canada (Oakville, Ontario). Radiolabeled precursors were diluted with unlabeled substrates to a specific activity of  $1 \mu\text{Ci}/\text{mmol}$ . The in vitro incubation conditions for the assay and analysis of the incorporation of the labeled precursors into fatty acids and glycerolipids were as described elsewhere (Stahl and Sparace, 1991; Kleppinger-Sparace et al., 1992). Standard reaction mixtures for acetate incorporation contained 0.25 M bis-tris-propane buffer (pH 7.9), 0.33 M sorbitol, 0.2 mM Na- $[1\text{-}^{14}\text{C}]\text{acetate}$ , 0.5 mM each of NADH, NADPH, and

CoA, and 1 mM  $\text{MnCl}_2$ , 6 mM each of  $\text{MgCl}_2$  and ATP, and 15 mM  $\text{KHCO}_3$ . Where indicated,  $[1\text{-}^{14}\text{C}]\text{acetate}$ , ATP, NADPH, and NADH in the standard mixture were omitted, replaced, or supplemented with other glycolytic intermediates. The effects of, or requirements for, oxidized nucleotides  $[\text{NAD(P)}^+]$  were not determined. The amount of plastid protein used for all treatments within each experiment generally ranged from 45 to 70  $\mu\text{g}$ . All experiments were performed at least twice. Data shown are the mean values of duplicate observations from representative experiments.

### Cytosolic Markers

Alcohol dehydrogenase, UDP-Glc pyrophosphorylase, and PPI:Fru-6-P-1-phosphotransferase were measured according to the method of Macdonald and ap Rees (1983), Borchert et al. (1993), and ap Rees et al. (1985), respectively.

## RESULTS AND DISCUSSION

### Incorporation of Glycolytic Intermediates

Under optimum conditions for fatty acid synthesis from acetate (Stahl and Sparace, 1991), pea root plastids readily incorporate radiolabeled pyruvate, Glc, and Glc-6-P into fatty acids (Table I). Pyruvate generally gave the greatest rates of fatty acid biosynthesis at all concentrations tested, whereas malate was relatively ineffective as a substrate for fatty acid synthesis. At low concentrations (0.5–2 mM), the rate of fatty acid synthesis from acetate was higher than that of all other labeled precursors except pyruvate. The rate of acetate incorporation was apparently saturated at 3 mM. However, the rates of pyruvate, Glc, Glc-6-P, and malate incorporation were still increasing somewhat at the highest concentration tested (5 mM). At this concentration, the rates of pyruvate, Glc, and Glc-6-P incorporation into fatty acids were 183, 155, and 125  $\text{nmol h}^{-1} \text{mg}^{-1}$  protein, respectively, compared to that for acetate and malate (99 and 57  $\text{nmol h}^{-1} \text{mg}^{-1}$  protein, respectively). On a nanomolar basis, pyruvate appears to be the preferred in vitro carbon donor for fatty acid biosynthesis. However, when one considers that each mole of hexose can give rise to 2 moles of pyruvate, Glc and Glc-6-P can both be considered more effective precursors. In any case, the results indicate that Glc, Glc-6-P, and pyruvate all can be metabolized through glycolysis and pyruvate dehydrogenase within pea root plastids to form acetyl-CoA, which is then incorporated into fatty acids. These observations support the idea that both hexokinase and phosphoglyceromutase are present in pea root plastids.

Our observations are in agreement with several related studies of other nonphotosynthetic plastids (Simcox et al., 1977; Journet and Douce, 1985; Kang and Rawsthorne, 1994) but are in contrast to two similar studies of pea root plastids. Borchert et al. (1993) and Trimming and Emes (1993) suggested that hexokinase and phosphoglyceromutase were not present in pea root plastids, based on comparisons with markers for cytosolic and mitochondrial contamination. There are two possible explanations for the apparent discrepancy between our results and those of

**Table 1.** Effects of various precursor concentrations on their utilization for fatty acid biosynthesis and the proportions of radioactive fatty acids synthesized by isolated pea root plastids

Plastids were incubated under optimum conditions for fatty acid synthesis from acetate (Stahl and Sparace, 1991). Where indicated, acetate was replaced with pyruvate, Glc, Glc-6-P, or malate. Only 0.5, 2, and 5 mM treatments were analyzed for fatty acid radioactivity.

| Precursor | Concentration | Incorporation                         | Fatty Acid Distribution |                |            |
|-----------|---------------|---------------------------------------|-------------------------|----------------|------------|
|           |               |                                       | Palmitic acid           | Stearic acid   | Oleic acid |
|           | mM            | nmol h <sup>-1</sup> mg <sup>-1</sup> |                         | %              |            |
| Acetate   | 0.5           | 38.1                                  | 32.7                    | 11.1           | 56.2       |
|           | 1             | 61.5                                  |                         |                |            |
|           | 2             | 93.5                                  | 29.3                    | 3.4            | 67.3       |
|           | 3             | 109.3                                 |                         |                |            |
|           | 5             | 99.0                                  | 29.6                    | 2.5            | 67.9       |
| Pyruvate  | 0.5           | 45.2                                  | 29.7                    | 4.5            | 65.8       |
|           | 1             | 79.3                                  |                         |                |            |
|           | 2             | 120.2                                 | 23.1                    | 2.3            | 74.6       |
|           | 3             | 162.3                                 |                         |                |            |
|           | 5             | 183.5                                 | 18.9                    | — <sup>a</sup> | 81.1       |
| Glc       | 0.5           | 24.6                                  | 33.0                    | 6.5            | 60.5       |
|           | 1             | 50.2                                  |                         |                |            |
|           | 2             | 80.3                                  | 26.3                    | 3.0            | 70.7       |
|           | 3             | 128.6                                 |                         |                |            |
|           | 5             | 154.9                                 | 20.5                    | —              | 79.5       |
| Glc-6-P   | 0.5           | 28.4                                  | 31.5                    | 2.6            | 65.9       |
|           | 1             | 50.6                                  |                         |                |            |
|           | 2             | 76.6                                  | 25.2                    | 3.7            | 71.1       |
|           | 3             | 113.8                                 |                         |                |            |
|           | 5             | 125.4                                 | 23.1                    | —              | 76.9       |
| Malate    | 0.5           | 13.5                                  | 38.6                    | 10.2           | 51.2       |
|           | 1             | 18.2                                  |                         |                |            |
|           | 2             | 26.58                                 | 33.1                    | 6.4            | 60.5       |
|           | 3             | 40.2                                  |                         |                |            |
|           | 5             | 57.3                                  | 30.5                    | —              | 69.5       |

<sup>a</sup> —, None detected.

enzymes that they measured were indeed very low, all were well in excess of those required for our observed rates of fatty acid biosynthesis.

To support our hypothesis that pea root plastids do in fact have their own complete glycolytic pathway, we also measured the activities of several cytosolic marker enzymes in our purified plastid preparations. This was necessary to rule out the possibility that contaminating glycolytic enzymes of the cytosol might be involved in the utilization of the various precursors for fatty acid synthesis. These markers were alcohol dehydrogenase, UDP-Glc pyrophosphorylase, and PPI:Fru-6-P-1-phosphotransferase. Although present in the crude cell-free homogenate (944, 1152, and 276 nmol min<sup>-1</sup> mg<sup>-1</sup> protein, respectively), these enzymes were not detected in the purified intact plastids or plastids lysed with 0.1% Triton X-100, indicating the absence of cytosolic contamination in our plastids. Furthermore, in an earlier study (Xue, 1993) the possibility of mitochondrial contamination as a source of pyruvate dehydrogenase was also ruled out. Therefore, we are confident that our observed incorporation of the various intermediates tested into fatty acids are due to enzymes naturally located within the plastid.

The utilization of Glc for fatty acid synthesis by pea root plastids at rates comparable to that for Glc-6-P raises an important question regarding the uptake of Glc by these plastids. It is known that Glc-6-P, but not Glc, is translocated into pea root plastids in counter-exchange with Pi by the phosphate translocator (Borchert et al., 1993). This would suggest that an uncharacterized translocator may exist for Glc. Alternatively, extraplastidic hexokinase may be preferentially bound to the surface of the root plastid as suggested by Trimming and Emes (1993), thus converting Glc to Glc-6-P prior to uptake. This latter explanation seems unlikely in view of the relatively high efficiency of Glc utilization and our own marker enzyme data.

The relatively low rates of malate utilization observed here are in agreement with those observed by Kang and Rawsthorne (1994) but in contrast to those observed by Smith et al. (1992), who found that malate was superior to both pyruvate and acetate for fatty acid biosynthesis in developing castor bean endosperm leukoplasts. They suggested that malate was metabolized to produce acetyl-CoA via the two sequential reactions of NAD(P)-linked malic enzyme and pyruvate dehydrogenase. Our results suggest that either the uptake of malate and/or malic enzyme activity is relatively low in pea root plastids, although not completely absent. Because of its relatively poor incorporation into fatty acids, however, the utilization of malate for fatty acid biosynthesis was not studied further except for the analysis of radioactive fatty acids synthesized.

It should be emphasized that the efficient utilization of the various glycolytic intermediates and malate used here for fatty acid biosynthesis indirectly requires a supply of oxidized nucleotides (NAD<sup>+</sup> and/or NADP<sup>+</sup>) as co-factors for glyceraldehyde-3-P dehydrogenase, pyruvate dehydrogenase, and NADP<sup>+</sup>-malic enzyme. In the present investigation, these are presumably derived from intraplastidic pools maintained by the reductive steps of de novo fatty

these latter workers. First, our observations were made with intact plastids, which are essential for de novo fatty acid biosynthesis, whereas those of Borchert et al. (1993) and Trimming and Emes (1993) were based on measurements of individual enzyme activities in most likely lysed plastid preparations. It is possible that the activities of these enzymes in lysed plastid preparations do not precisely or directly reflect their activities in the intact plastid. Second, as pointed out by Trimming and Emes (1993), different cytosolic markers can lead to slightly different interpretations regarding estimates of cytosolic contamination and net enzymic activities associated with various plastid preparations. This is particularly a problem when dealing with plastidic enzymes having low activities and recoveries close to those of the markers. It is interesting to note that Trimming and Emes (1993) also pointed out that, although the specific activities of some of the glycolytic

acid biosynthesis. It is possible that fatty acid biosynthesis from the various precursors used in this study might be improved by exogenously supplied oxidized nucleotides. However, the effects of oxidized nucleotides were not determined here.

### Labeling Patterns of Fatty Acids

Fatty acids synthesized by pea root plastids from all labeled precursors tested were exclusively palmitate, stearate, and oleate (Table I). However, the distribution of radioactivity among labeled fatty acids was affected by different precursor concentrations. Higher concentrations (2 and 5 mM) of each precursor caused an increase in the proportion of radiolabeled oleate accumulated with corresponding decreases in the proportions of palmitate and stearate. These observations are in agreement with our previous studies (Kleppinger-Sparace et al., 1992; Qi et al., 1994). The apparent promotion of palmitate elongation and stearate desaturation by these precursors may be due to an increase in the intraplastidic levels of NAD(P)H and/or acetyl-CoA or malonyl-CoA resulting from the glycolytic metabolism of these intermediates and malate. Increased levels of reduced nucleotides and fatty acid precursors would favor fatty acid elongation (Stumpf, 1984; Harwood, 1988).

### Dependency on ATP and Reduced Nucleotides

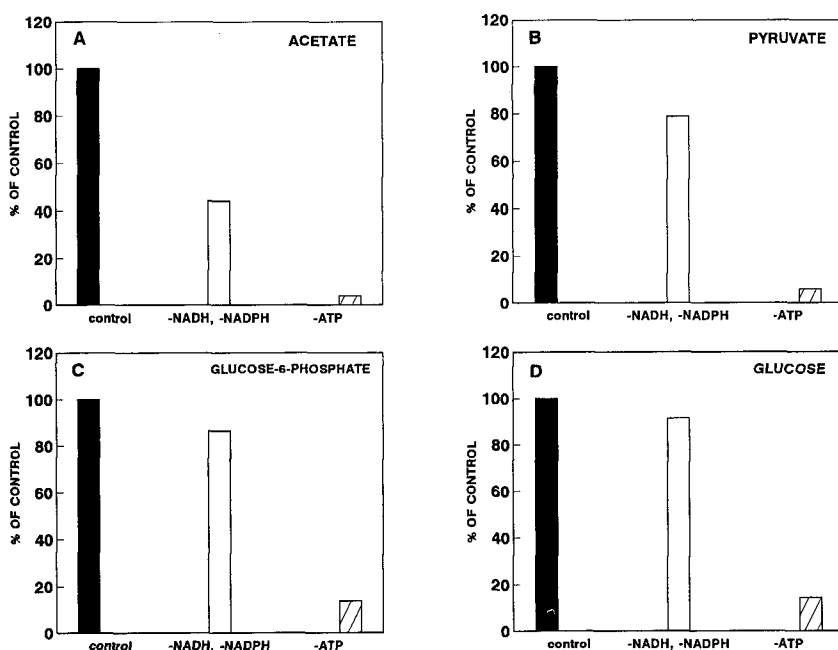
The utilization of acetate, Glc, Glc-6-P, and pyruvate for fatty acid biosynthesis was variously dependent on the exogenously supplied high-energy co-factors, ATP and NAD(P)H (Fig. 1). When [1-<sup>14</sup>C]acetate was used as the precursor, ATP, NADH, and NADPH were required to maximize the rate of fatty acid synthesis (Fig. 1A). The deletion of both reduced nucleotides or ATP from the

reaction mixture resulted in 56 or 96% decreases, respectively, in the rates of fatty acid synthesis from acetate. In contrast, however, when pyruvate, Glc-6-P, and Glc were used as precursors, the combined deletion of NADH and NADPH caused only an approximately 10 to 20% decrease in the incorporation of these precursors (Fig. 1, B-D). These observations indicate that the metabolism of these precursors via plastidic glycolysis or the oxidative pentose phosphate pathway results in the production of much of the reduced nucleotides required for fatty acid synthesis by pea root plastids, thus partially alleviating the requirement for exogenously supplied NAD(P)H. This would be achieved at the levels of glyceraldehyde-3-P dehydrogenase in glycolysis (Trimming and Emes, 1993), pyruvate dehydrogenase (Camp and Randall, 1985; Denyer and Smith, 1988; Dennis, 1989), and Glc-6-P and 6-phosphogluconate dehydrogenases in the oxidative pentose phosphate pathway (Bowsher et al., 1989, 1992; Borchert et al., 1993). These results support the idea discussed earlier that the plastids used in this investigation do contain a pool of oxidized nucleotides to promote the utilization of radioactive precursors. Our observations are in agreement with a similar study of plastids from developing castor seed endosperm (Smith et al., 1992).

The omission of reduced nucleotides consistently caused approximately a 10% reduction in the amounts of stearate and oleate accumulated, with a corresponding increase in the amount of palmitate (Table II). These observations are consistent with the idea that intraplastidic levels of NAD(P)H, as modulated by plastidic glycolysis, can affect the rates of fatty acid elongation and desaturation as mentioned earlier.

Fatty acid biosynthesis from Glc, Glc-6-P, and pyruvate was still greatly dependent (85–95%) on exogenously supplied ATP, as normally observed with acetate as the pre-

**Figure 1.** Effects of omitting NAD(P)H and ATP on the rates of fatty acid synthesis from various radiolabeled precursors by pea root plastids. The concentration of each radiolabeled precursor was 2 mM. Control reactions were carried out in the presence of 6 mM ATP and 0.5 mM each of NADH and NADPH. The control rates of fatty acid synthesis from acetate, pyruvate, Glc-6-P, and Glc were 83, 122.5, 74.1, and 99.1 nmol h<sup>-1</sup> mg<sup>-1</sup> protein, respectively, which were set at 100%.



**Table II.** Effects of omitting NAD(P)H on the compositions of radioactive fatty acids synthesized from various precursors by isolated pea root plastids

Data shown are from analyses of total fatty acids synthesized in selected treatments shown in Figure 2.

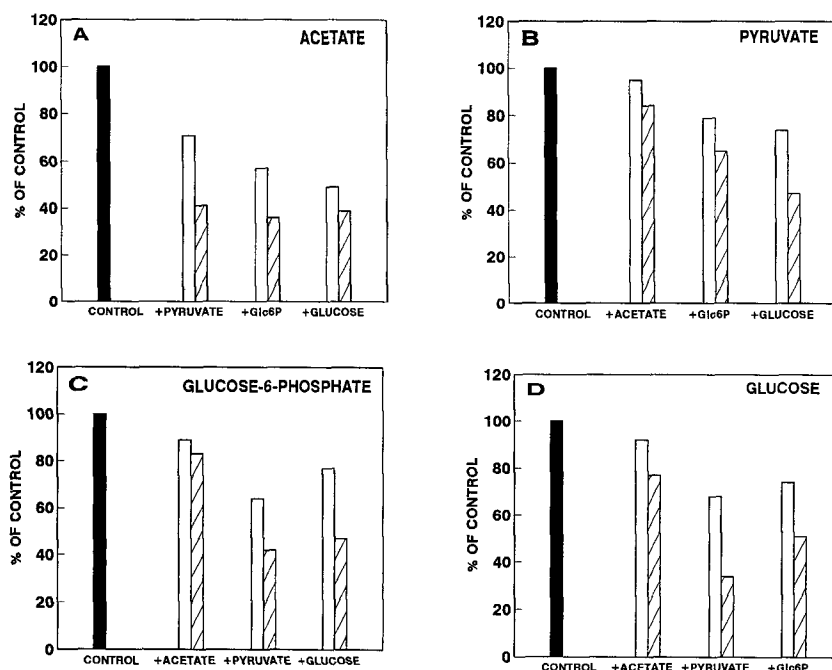
| Treatment          | Incorporation<br><i>nmol h<sup>-1</sup> mg<sup>-1</sup></i> | Fatty Acid Distribution |              |            |
|--------------------|---|-------------------------|--------------|------------|
|                    |   | Palmitic acid           | Stearic acid | Oleic acid |
|                    |   | %                       |              |            |
| Acetate control    | 83.0  | 35.1                    | 9.1          | 55.8       |
| Acetate, -NAD(P)H  | 36.5  | 46.2                    | 5.4          | 48.4       |
| Pyruvate control   | 122.5   | 24.0                    | 3.9          | 72.1       |
| Pyruvate, -NAD(P)H | 96.7  | 35.2                    | 8.1          | 56.7       |
| Glc control        | 99.1  | 27.2                    | 6.1          | 66.7       |
| Glc, -NAD(P)H      | 90.6  | 36.3                    | 2.7          | 61.0       |
| Glc-6-P control    | 74.1  | 25.8                    | 5.2          | 69.0       |
| Glc-6-P, -NAD(P)H  | 64.0  | 34.3                    | 3.7          | 62.0       |

cursor (Fig. 1). The results with pyruvate are somewhat expected, since the metabolic conversion of this precursor into fatty acid does not involve any ATP-producing reactions. However, the results obtained with Glc and Glc-6-P are somewhat surprising, since the glycolytic catabolism of these hexoses is expected to yield stoichiometric amounts of ATP required for fatty acid biosynthesis at the levels of PGA and pyruvate kinases. Instead, with either of these precursors the dependence of fatty acid synthesis on exogenously supplied ATP is reduced only to approximately 86% as compared to 96 and 94% dependency for acetate and pyruvate, respectively. These observations indicate that the complete glycolytic pathway of pea root plastids

provides only a small proportion of the ATP required for fatty acid biosynthesis, which is in agreement with our previous results (Qi et al., 1994). In contrast, the energy yield of PGA kinase can be greatly improved when it functions as part of the triose phosphate shuttle (Kleppinger-Sparace et al., 1992; Qi et al., 1994). The simplest explanation for the apparent discrepancy between the energy yield of the complete glycolytic pathway and the ATP required for fatty acid biosynthesis is that other ATP-dependent processes such as the biosynthesis of starch, protein, nucleic acids, or polyprenoids may be occurring in pea root plastids in addition to fatty acid biosynthesis. That these processes are occurring is likely, since these plastids are isolated from rapidly growing germinating pea root tips. However, the extent to which these processes are occurring in our plastids is presently not known.

### Effects of Cold Precursors

In competition experiments, 5 mM each of unlabeled pyruvate, Glc-6-P, and Glc all reduced [<sup>14</sup>C]acetate incorporation by up to 60% (Fig. 2A). Similarly, the amounts of radiolabel from [<sup>14</sup>C]Glc-6-P and [<sup>14</sup>C]Glc incorporated into fatty acids by pea root plastids were reduced by 58 and 66%, respectively, upon co-incubation with 5 mM unlabeled pyruvate (Fig. 2, C and D). Glc inhibition of [<sup>14</sup>C]Glc-6-P incorporation and Glc-6-P inhibition of [<sup>14</sup>C]Glc incorporation were also observed. Overall, 5 mM cold pyruvate generally had the greatest inhibitory effects on the utilization of labeled precursors, whereas cold acetate had the least inhibitory effects. Similar, but reduced, effects were observed when 0.5 mM of each cold precursor was tested. Such apparent reductions in the utilization of radiolabeled precursors for fatty acid biosynthesis by the various cold precursors would be expected if they were all part of the



**Figure 2.** Effects of cold substrate competition on fatty acid synthesis from radiolabeled precursors by isolated pea root plastids. The concentration of all radiolabeled precursors in all treatments was 2 mM. Controls (black bars) did not contain any cold precursors and their rates of utilization for fatty acid synthesis were 91.3, 169.9, 106.1, and 114.1  $\text{nmol h}^{-1} \text{mg}^{-1}$  protein, respectively, for radiolabeled acetate, pyruvate, Glc-6-P, and Glc. The concentrations of cold precursors tested were 0.5 mM (open bars) and 5 mM (cross-hatched bars).

same functioning metabolic pathway. However, the amounts of each reduction would greatly depend on the pool sizes of these metabolites within the plastid as well as their individual rates of uptake. In the simplest sense, these observations support the idea that the complete pathway from Glc via glycolysis to fatty acids is present in pea root plastids and that pyruvate may be a more accessible or more direct precursor for fatty acid biosynthesis.

### Labeling Patterns of Glycerolipids

Fatty acids synthesized from labeled acetate, pyruvate, Glc-6-P, and Glc by pea root plastids are accumulated in a variety of glycerolipids under standard incubation conditions (Table III). These are primarily PA, PC, PG, DAG, and TAG. The most notable differences among the various precursors tested was that acetate utilization resulted in a relatively unique distribution pattern of some lipids accumulated in comparison to the other precursors. With acetate, approximately 7 to 10% more PA and 3 to 5% more PG accumulated, and approximately 4 and 8% less, respectively, of TAG and PC accumulated than observed with the other precursors. These observations lead us to speculate that PA phosphatase in these plastids may be one of the enzymes of lipid metabolism most affected. When Glc, Glc-6-P, and pyruvate are used as precursors, this enzyme may be enhanced, thus leading to an increase in PC and TAG accumulation and a decrease in PG accumulation. However, the activity of PA phosphatase was not directly measured in these experiments to confirm this possibility.

In summary, the data presented here demonstrate that isolated pea root plastids can readily utilize Glc, Glc-6-P, and pyruvate as carbon sources for fatty acid biosynthesis at rates comparable to or greater than acetate. Our observations with Glc and Glc-6-P argue in favor of a complete glycolytic pathway in pea root plastids that provides carbon and reduced nucleotides for fatty acid biosynthesis in these plastids. This would necessarily include the presence of sufficient, albeit relatively low, activities of hexokinase

and phosphoglyceromutase. Furthermore, the metabolism of these glycolytic intermediates may also modulate fatty acid and glycerolipid biosynthesis in two possible ways. First, by increasing pool sizes or availability of acetyl-CoA, malonyl-CoA, and/or NAD(P)H, the reactions of fatty acid elongation and DAG-dependent lipid biosynthesis may be favored. Second, some intermediates of glycolytic metabolism may exert regulatory effects on the activity of other key enzymes associated with fatty acid synthesis. In particular, our unpublished observations indicate that PEP and Fru-1,6-diphosphate both can stimulate acetyl-CoA carboxylase and that dihydroxyacetone phosphate causes an increase in activity of pyruvate dehydrogenase in pea root plastids. We are currently investigating the role that these enzymes may have in regulating the flow of glycolytic carbon into fatty acid biosynthesis in these plastids.

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**Table III.** Effects of various precursors on the distribution of radioactivity among lipids synthesized by pea root plastids

Data shown are derived from analysis of replicate 2-mm treatments from Table I.

| Lipid               | Precursor Distribution |          |         |      |
|---------------------|------------------------|----------|---------|------|
|                     | Acetate                | Pyruvate | Glc-6-P | Glc  |
|                     | %                      |          |         |      |
| TAG                 | 9.6                    | 13.6     | 12.2    | 13.4 |
| DAG                 | 17.5                   | 19.5     | 18.7    | 18.2 |
| MAG                 | 1.5                    | 2.5      | 1.9     | 3.0  |
| FFA                 | 6.0                    | 7.2      | 7.6     | 5.8  |
| PA                  | 23.1                   | 13.4     | 15.7    | 14.8 |
| PG                  | 13.0                   | 10.6     | 9.2     | 8.4  |
| PC                  | 18.9                   | 25.0     | 27.4    | 27.5 |
| PI                  | 2.8                    | 1.9      | 2.5     | 2.1  |
| Others <sup>a</sup> | 7.6                    | 6.3      | 4.8     | 6.8  |

<sup>a</sup> Others correspond to unidentified regions at TLC solvent front and origins.

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