β-Carotene Synthesis in Isolated Spinach Chloroplasts

ITS TIGHT LINKAGE TO PHOTOSYNTHETIC CARBON METABOLISM

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ABSTRACT
Carefully isolated intact spinach chloroplasts virtually free of contamination of other organelles effectively form β-carotene from NaH¹⁴CO₃ or [U-¹⁴C]-3-phosphoglycerate (PGA) under photosynthetic conditions. The photosynthes pool formed in chloroplasts from 1 to 2 millimolar [U-¹⁴C]-3-PGA or 3 to 6 millimolar NaH¹⁴CO₃ was fully sufficient to supply β-carotene synthesis with intermediates for about 1 hour at maximal rates of about 20 nanomoles ¹⁴C incorporated per milligram chlorophyll per hour. Fatty acid synthesis remains, under these circumstances, in linear dependence to substrate concentrations with far lower activity. Isotopic dilution of the β-carotene synthesis by adding unlabelled glyceraldehyde 3-phosphate, dihydroxyacetone-P, 3-PGA, 2-PGA, phosphoenolpyruvate, pyruvate, respectively, may be interpreted as a direct substrate flow from photosynthetically fixed CO₂ to isopentenyl pyrophosphate fixing system. Unlabelled acetate did not dilute β-carotene synthesis. Fatty acid synthesis acted similarly with unlabelled substrates; but it was also diluted by unlabelled acetate. These results indicate a tight linkage of photosynthetic carbon fixation and plastid isoprenoid synthesis.

Studies in the laboratory of TW Goodwin (9) on seedlings from several species and investigations on spinach protoplasts (24) doubtless demonstrated the existence of two separate pathways for isoprenoid synthesis in higher plants; one for an extraplastidic site forming sterols and another for the chloroplasts forming plastid isoprenoids such as carotenoids. The synthesis of carotene and other plastid terpenoids from mevalonate acid is well established from experiments using isolated chloroplasts (5, 6). Detection of acetocetetyl-CoA formation (23) and identification of enzymes in the chloroplast like HMG-CoA reductase (1, 31), mevalonate kinase (1), and 5-phosphomevalonate kinase (1) are consistent with the generally accepted view that chloroplast isoprenoids are synthesized from acetyl-CoA via acetocetetyl-CoA, HMG-CoA, and mevalonate. Only a few results are available about the sequence from photosynthetically fixed CO₂ to plastid isoprenoids isolated intact chloroplasts, e.g. from spinach (3, 11) and from Acetabularia (18).

In this study we demonstrate the preferential formation of β-carotene compared to fatty acids from NaH¹⁴CO₃ or [U-¹⁴C]-3-PGA in isolated spinach chloroplasts. Intriguing results were achieved by applying substrates which are in all probability involved in a pathway from photosynthetically fixed carbon to acetyl-CoA using the isotopic dilution method. The partly different isotopic dilutions found in the synthesis of β-carotene and fatty acids are discussed and a model is introduced.

MATERIALS AND METHODS

Chemicals. Sodium salts of ¹⁴C-bicarbonate (2.00 GBq mmol⁻¹), [¹⁴C]pyruvic acid (0.36 GBq mmol⁻¹), [²-¹⁴C]pyruvic acid (0.46 GBq mmol⁻¹); cyclohexylammonium salts of phos- phoenol[¹⁴C]pyruvic acid (1.18 GBq mmol⁻¹) and [¹⁴C]-3-PGA (4.96 GBq mmol⁻¹). [¹⁴C]leucine (12.65 GBq mmol⁻¹) were obtained from Amersham Buchler, Braunschweig, F.R.G. The TLC plates precoated with silicagel G1500, LS 254 were purchased from Schleicher & Schuell, Einbeck, F.R.G. All other biochemicals and solvents were of highest analytical grade and were obtained from Sigma, St. Louis, MO, and E. Merck, Darmstadt, F.R.G.

Plant Material and Chloroplast Preparation. Leaves of 3 to 4 weeks old field grown spinach (Spinacia oleracea, var Butterfly) were used immediately after picking for all experiments. Intact chloroplasts were isolated according to Jensen and Bas- sham (14) (A) or to Nakatani and Barber (20) (B) with the following modifications. For (A), after preillumination with white light (0.06 J cm⁻² s⁻¹) for 50 min at 4°C, washed and deribbed leaves were homogenized in isotonic medium (pH 6.5) (containing 330 mM sorbitol; 2 mM EDTA; 4 mM ascorbate; 1 mM MnCl₂; 2 mM MgCl₂; 0.5 mM KH₂PO₄; 20 mM NaCl; 4 mM cysteine and 50 mM Mes-buffer, adjusted with 1 M KOH to pH 6.5) for 4 × 1 s using a Waring Blender. The homogenate was filtered through a 20 μm nylon gauze (Vereinigte Seidenwebereien, Krefeld, F.R.G.) and centrifuged for 1 min at 2100g. The pellet was washed in a resuspending medium (containing 330 mM sorbitol; 2 mM NaNO₃; 2 mM EDTA; 4 mM ascorbate; 1 mM MnCl₂; 2 mM MgCl₂; 0.5 mM KH₂PO₄; 20 mM NaCl and 50 mM Hepes-buffer, adjusted with 1 M KOH to pH 7.6) three times and centrifuged for 1 min at 1500g. The same procedure was used for (B) but the isolation medium (containing 330 mM sorbitol; 0.2 mM MgCl₂; 20 mM Mes-buffer, adjusted with 1 M KOH to pH 6.5) and the resuspending medium (containing 330 mM sorbitol; 0.4 mM MgCl₂; 50 mM Hepes-buffer, adjusted with 1 M KOH to pH 7.6) contains low amounts of MgCl₂ as mineral compound. The resulting suspensions of intact chloroplasts were used for the experiments. In some cases the chloroplast suspension was placed on a linear 0 to 80% Percoll (Pharmacia, Uppsala, Sweden) gradient described in Schulze-Siebert et al. (22), but since this increased the preparation time without improving the results, this purification step was not routinely followed.

1 Supported by the Deutsche Forschungsgemeinschaft.

2 Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; DHAP, dihydroxyacetone phosphate; E-4-P, erythrose 4-phosphate; GAP, glyceraldehyde 3-phosphate; IPP, isopentenyl pyrophosphate; (non) reversible GAPDH, (non) reversible NADP-d-glyceraldehyde 3-phosphate dehydrogenase; PEP, phosphoenolpyruvate; 3-PGA, 3-phosphoglycerate; 2-PGA, 2-phosphoglycerate.

The chloroplasts were isolated as described above and used for experiments immediately. The photosynthetic pool formed in chloroplasts from 1 to 2 millimolar [U-¹⁴C]-3-PGA or 3 to 6 millimolar NaH¹⁴CO₃ was fully sufficient to supply β-carotene synthesis with intermediates for about 1 hour at maximal rates of about 20 nanomoles ¹⁴C incorporated per milligram chlorophyll per hour. Fatty acid synthesis remains, under these circumstances, in linear dependence to substrate concentrations with far lower activity. Isotopic dilution of the β-carotene synthesis by adding unlabelled glyceraldehyde 3-phosphate, dihydroxyacetone-P, 3-PGA, 2-PGA, phosphoenolpyruvate, pyruvate, respectively, may be interpreted as a direct substrate flow from photosynthetically fixed CO₂ to isopentenyl pyrophosphate fixing system. Unlabelled acetate did not dilute β-carotene synthesis. Fatty acid synthesis acted similarly with unlabelled substrates; but it was also diluted by unlabelled acetate. These results indicate a tight linkage of photosynthetic carbon fixation and plastid isoprenoid synthesis.
**Reaction Mixtures.** If not specified, the standard reaction mixture of intact chloroplasts contained in a final volume of 0.5 ml: 330 mM sorbitol; 2 mM NaNO₃; 2 mM EDTA; 4 mM ascorbate; 1 mM MnCl₂; 2 mM MgCl₂; 0.5 mM KH₂PO₄; 20 mM NaCl; 0.05 mM glutamate; 1 mM oxaloacetic acid; 50 mM Hepes-buffer adjusted with 1 mM KOH to pH 7.6 and chloroplasts equivalent to 80 to 100 µg of Chl per ml. As compared to control oxaloacetate, when added at a concentration of 1 mM, this increases formation of β-carotene, fatty acids, and so forth about twice (U Homeyer, G. Schultz, unpublished data). After preillumination of 3 min the reactions were started by adding the radioactive substrates. The mixtures were kept at 20 ± 2°C in a water bath in the light (0.2 J cm⁻² s⁻¹). In time course experiments the volume was quadrupled. Aliquots of 0.5 ml were taken at indicated times.

**Assay of Isoprenoids and Fatty Acids.** The aliquots of 0.5 ml were transferred into 0.6 ml chloroform:methanol (1:2,v/v) and then 0.25 ml chloroform and 0.5 ml water; 100 µg β-carotene and PQ each and 300 µg fatty acid were added as carriers. Identification of β-carotene, plastoquinone, and fatty acids as its fatty acid methyl esters were performed by repeated adsorption and partition chromatography as described in Schulze-Siebert and Schultz (24). For determination of radioactivity the corresponding zones were scraped out and measured in 1 ml methanol plus 4 ml Hydroluma (Baker Chemicals, Deventer, The Netherlands) by scintillation counting (Packard Tricarb 3255). The recovery rates of β-carotene (35%) and fatty acids (50%) were determined by using internal reference substances and were taken into consideration when calculating the incorporation rates.

**Other Methods.** CO₂-fixation rates of the isolated chloroplasts were determined by the following method. The reaction mixture, plus 5 mM NaH¹⁴CO₃, was illuminated and after 0, 2, 4, and 6 min two 25 µl aliquots were taken. One aliquot was transferred into 300 µl triethanolamine-KOH buffer (pH 9.0). The parallel aliquot was treated with 300 µl 6 M HCl and perfused with a stream of N₂ to remove dissolved CO₂. The CO₂-fixation rate was calculated from differences of ¹⁴CO₂ contents of HCl treated samples taken at 0 to 2 and 4 min. Chl contents were determined according to the method of Arnon (2), and enzymes as described in the following references: hydroxypruvate reductase (EC 1.1.1.81) by Tolbert (29); nonreversible GAPDH (EC 1.2.1.9) and reversible GAPDH (EC 1.2.1.13) by Kelly and Gibbs (16) and shikimate oxidoreductase (EC 1.1.1.25) by Fiedler and Schultz (8).

**RESULTS AND DISCUSSION**

**Characterization of Isolated Chloroplasts.** Chloroplast intactness of 83 to 92% were measured by plasticid marker enzymes as reversible GAPDH (EC 1.2.1.13) (16) and shikimate oxidoreductase (EC 1.1.1.25) (8) in the same manner as described in (24). The CO₂-fixation rate of intact chloroplasts were 80 to 120 µmol CO₂ h⁻¹ mg⁻¹ Chl.

The rates of fatty acid and β-carotene synthesis were linear over a period of 60 min (Fig. 1). Compared to chloroplasts isolated according to a modified method of Jensen and Basham (14), a significantly lower incorporation from ¹⁴CO₂ into β-carotene was found in chloroplasts isolated according to Nakatani and Barber (20), although no differences could be found in fatty acid synthesis between the two chloroplast preparations. Purification of chloroplasts over a Percoll gradient neither activated nor inactivated fatty acid and β-carotene formation (Fig. 1). For this reason chloroplasts were isolated according to the modified method of Jensen and Basham and the percoll gradient purification was omitted in the following experiments. These chloroplasts were slightly contaminated with 1 to 2% peroxisomes and less than 1% cytosol when related to the total amount per mg Chl in the leaf homogenate. The marker enzymes were

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![Fig. 1. Time course of the formation of β-carotene (•), fatty acids (□, △) from NaH¹⁴CO₃ in intact spinach chloroplasts isolated by different methods: modified according to Jensen and Basham (14) (•, □), the same but additionally purified over a Percoll gradient (22) (□, △) (78 µmol CO₂ fixed mg⁻¹ Chl h⁻¹); modified according to Nakatani and Barber (20) and purified over Percoll gradient (△, △) (68 µmol CO₂ fixed mg⁻¹ Chl h⁻¹). Chloroplasts (0.12 mg Chl ml⁻¹) were illuminated in the standard reaction mixture containing 5 mM NaH¹⁴CO₃. Aliquots were taken at times indicated and the reaction was terminated as described in "Materials and Methods." Results are expressed as nmol ¹⁴CO₂ incorporated into β-carotene or fatty acids.](image-url)

<table>
<thead>
<tr>
<th>¹⁴CO₂ Incorporation into</th>
<th>β-carotene</th>
<th>Fatty acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Control</td>
<td>37.1</td>
<td>7.8</td>
</tr>
<tr>
<td>(b) 3-PGA</td>
<td>7.8 (21)*</td>
<td>3.3 (42)</td>
</tr>
<tr>
<td>1 mM</td>
<td>5.9 (16)</td>
<td>2.7 (34)</td>
</tr>
<tr>
<td>5 mM</td>
<td>27.1 (73)</td>
<td>6.3 (81)</td>
</tr>
<tr>
<td>(c) 2-PGA</td>
<td>24.5 (66)</td>
<td>5.9 (75)</td>
</tr>
<tr>
<td>1 mM</td>
<td>6.7 (18)</td>
<td>3.4 (44)</td>
</tr>
<tr>
<td>5 mM</td>
<td>4.8 (13)</td>
<td>2.3 (30)</td>
</tr>
</tbody>
</table>

* % of control.

Table 1. Isotopic Dilution Experiment

Indirect test by an isotopic dilution experiment (NaH¹⁴CO₃ against 3-PGA [in b] or 2-PGA [in c]) whether cytosolic phosphoglycerate mutase (PGM) contaminates the chloroplast preparation used: When in (b) contaminating cytosolic PGM were present, 3-PGA would be formed from added 2-PGA and thus incorporated ¹⁴CO₂ would be diluted by 3-PGA imported by the phosphate translocator. In this case the results in (c) with 2-PGA should coincide with that in (b) with 3-PGA. The dilution effect of 2-PGA, when transformed to 3-PGA by added PGM, is shown in (d). As the results in (a) and (c) compared to (b) and (d) indicate the contamination of chloroplast suspension by cytosolic PGM is unimportant. Intact spinach chloroplasts were illuminated in the standard reaction mixture containing 5 mM NaH¹⁴CO₃ and unlabeled 3-PGA, 2-PGA, and 2-PGA plus added PGM at concentrations indicated. Prior to use the purchased PGM (rabbit) was desalted through Sephadex G-25. After a period of 30 min the reaction was terminated as described in "Materials and Methods."

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contamination of cytosol in these chloroplast suspensions were considerable. 2-PGA should be isomerized into 3-PGA by cytosolic phosphoglycerate mutase and no difference would be found between the isotopic dilution with 3-PGA and 2-PGA. Such a behavior was only observed when phosphoglycerate mutase (Böhringer, Mannheim, F.R.G.; desalted by a small G-25 column) was exogenously added with 2-PGA. These results show that the chloroplasts used were virtually free of cytosol. The slight decrease of \(^{14}\text{C}\)-incorporation by adding unlabeled 2-PGA will be dealt in the following section.

Synthesis of \(\beta\)-carotene and Fatty Acids from Exogenously Added \(\text{NaH}^{14}\text{CO}_3\) and 3-Phospho[\(\text{U-}\text{\textsuperscript{14}C}\)]-3-PGA and \(\text{NaH}^{14}\text{CO}_3\) were added to illuminated chloroplasts in the standard reaction mixtures (pH 7.6). Of the acetyl derived compounds preferentially \(\beta\)-carotene was formed (Fig. 2). One to 2 mM of [\(\text{U-}\text{\textsuperscript{14}C}\)]-3-PGA added as a substitute for the photosynthesate pool was fully sufficient to supply \(\beta\)-carotene synthesis at maximal rates with intermediates for about more than 0.5 h. This suggested \(\beta\)-carotene formation is a system of high affinity to intermediate substrates (Fig. 2a).

In contrast, enzymes of fatty acid formation exhibit a far lower affinity to intermediate substrates. Applying 0.1 to 1 mM 3-PGA (Fig. 2a) the rate of fatty acid synthesis was 5- to 8-fold lower than carotene formation. The rate linearly increased with higher concentrations but did not reach its maximal rate known from adding acetate as substrate (19; 24; 25).

For incorporation of \(\text{NaH}^{14}\text{CO}_3\), 3 to 6 mM is needed as substrate to reach a pool size of photosynthetic which was sufficient to supply \(\beta\)-carotene synthesis with intermediates for running at maximal rates for at least 0.5 h (Fig. 2b). The substrate concentration of 3 to 6 mM \(\text{NaH}^{14}\text{CO}_3\) may be equivalent to 1 to 2 mM of 3-PGA if one assumes that 1 mol 3-PGA is formed from 3 mM \(\text{NaHCO}_3\). Fatty acid synthesis behaves just as with 3-PGA as substrate. The rate of fatty acid formation from \(\text{NaH}^{14}\text{CO}_3\) was not unlike earlier work (19) and increased linearly in the presence of increasing concentrations of substrates but stayed far behind that of \(\beta\)-carotene under conditions tested.

The kinetics observed in \(\beta\)-carotene and fatty acid synthesis are apparently in accordance with known apparent Michaelis constants \(K_m\) for enzymes involved in respective syntheses. The affinity to intermediates in \(\beta\)-carotene may be reflected in \(K_m\) values of the sum of enzymes involved in respective syntheses. The lower affinity to intermediates of fatty acid synthesis may be attributed to high apparent \(K_m\) values of involved enzymes. These are 10- to 100-fold higher than those for \(\beta\)-carotene formation (13, 21, 30).

Isotopic Dilution of \(\text{NaH}^{14}\text{CO}_3\) with Unlabeled Compounds. When \(\text{NaH}^{14}\text{CO}_3\) was applied simultaneously with unlabeled compounds to spinach chloroplasts, an isotopic dilution effect of incorporated \(^{14}\text{C}\) was expected (Fig. 3). In the case of \(\beta\)-carotene (Fig. 3a) the rate of \(^{14}\text{C}\)-incorporation was diminished with increasing amounts of triose-P (GAP, DHAP) and 3-PGA. A decrease of about 80% was obtained after adding 5 mM unlabeled substrate. A smaller decrease of only 30% was found with unlabeled pyruvate, PEP, and 2-PGA. An inhibition of synthesis by higher amounts of added unlabeled substrates, at least of added 3-PGA, can be excluded from results as in Figure 2.

The lower isotopic dilution using 2-PGA, PEP, and pyruvate may be attributed to a relatively slow transport across the chloroplast envelope membrane. This may be caused by the lower specificity of the phosphate translocator for transport of 2-PGA and PEP (12) or by slow diffusion rate in the case of pyruvate (22). To eliminate transport barriers, chloroplasts were mechanically disrupted or osmotically shocked. But these chloroplasts

![Fig. 2. Incorporation of \(\text{NaH}^{14}\text{CO}_3\) and [\(\text{U-}\text{\textsuperscript{14}C}\)]-3-PGA into \(\beta\)-carotene and fatty acids of isolated spinach chloroplasts. Intact chloroplasts isolated modified according to Jensen and Bassham (14) (98 \(\mu\text{mol CO}_2\) fixed \(\text{mg}^{-1} \text{Chl h}^{-1}\)) were applied to the standard reaction mixture and started with concentrations of [\(\text{U-}\text{\textsuperscript{14}C}\)]-3-PGA (2a) or \(\text{NaH}^{14}\text{CO}_3\) (2b) as indicated. After 30 min of illumination the reaction was terminated as described in "Material and Methods." Results are expressed as nanomoles \(^{14}\text{C}\) incorporated into \(\beta\)-carotene (O) and fatty acid (C).](https://example.com/fig2.png)

![Fig. 3. Isotopic dilution of \(\text{NaH}^{14}\text{CO}_3\) by unlabeled compounds. Isolated chloroplasts were illuminated in the standard reaction mixture containing 5 mM \(\text{NaH}^{14}\text{CO}_3\) and unlabeled compounds at concentrations indicated. After a period of 30 min the reaction was terminated as described in "Material and Methods." The \(^{14}\text{C}\)-incorporation from \(\text{NaH}^{14}\text{CO}_3\) without adding unlabeled compounds were: for \(\beta\)-carotene 14 mmol h\(^{-1}\) mg\(^{-1}\) Chl (=100% in a) for fatty acids 6 mmol h\(^{-1}\) mg\(^{-1}\) Chl (=100% in b). The unlabeled compounds added were: acetate (■), 2-PGA (●), PEP (△), pyruvate (△), GAP (●), DHAP (●), 3-PGA (●).](https://example.com/fig3.png)
showed little or no incorporation of precursors (data not shown) because probably internal plastidic structures were destroyed. On the other hand, triosephosphates and 3-PGA are known to be transported at high rates into the chloroplast by the phosphate translocator which is very specific for these compounds (12).

The same isotopic dilution effects were found in fatty acid formation (Fig. 3b) but, additionally, acetate was as effective as triosephosphates and 3-PGA and, therefore, seems to be translocated at higher rates than 2-PGA, PEP, and pyruvate.

An interesting result was obtained when NaH\(^{14}\)CO\(_3\) in the presence of increasing amounts of unlabeled acetate were applied and \(\beta\)-carotene formation was followed. No isotopic dilution could be observed. This pointed to the existence of a direct substrate flow from CO\(_2\)-fixation to plastidic isoprenoid synthesis in chloroplasts. We suggest that this pathway is so tightly linked to photosynthetic carbon fixation that acetyl-CoA formed from added unlabeled acetate is ineffective in dilution of \(\beta\)-carotene. However, this metabolic channeling of photosynthetically fixed CO\(_2\) to \(\beta\)-carotene formation is only a relative one. This may be concluded from the following three points:

First, DHAP, GAP, and 3-PGA, which influence directly the 3-PGA pool of the chloroplast, diminished incorporation of \(^{14}\)C from NaH\(^{14}\)CO\(_3\) into \(\beta\)-carotene by isotopic dilution effects. Similar results were obtained with 2-PGA, PEP, and pyruvate. All these compounds could be channeled into the tightly linked pathway.

Second, when [\(^2\)\(^-\)\(^{14}\)C]pyruvate (3 mM) (but not [\(^1\)\(^-\)\(^{14}\)C]PEP or [\(^1\)\(^-\)\(^{14}\)C]pyruvate) were applied to chloroplasts, the radioactive label could be detected in \(\beta\)-carotene and fatty acids. This indicates a metabolization of these labeled compounds and an intermediate formation of [\(^1\)\(^-\)\(^{14}\)C]acetyl-CoA at the pyruvate dehydrogenase complex. However, since uptake by diffusion of pyruvate is slow (22), the incorporation of \(^{14}\)C from labeled pyruvate into carotene (5 nmol \(^{14}\)C atoms h\(^{-1}\) mg\(^{-1}\) Chl) was lower than that from NaH\(^{14}\)CO\(_3\) (Fig. 2b).

Third, when NaH\(^{14}\)CO\(_3\) is absent, the acetyl-CoA-pool formed from exogenous acetate also supplies the plastidic isoprenoid synthesis (11, 24).

To counter arguments that bypasses may be involved, like leucine in carotene synthesis of *Phycymyes blakesleeanus* (10), [\(^{1}\)\(^-\)\(^{14}\)C]leucine was applied to illuminated chloroplasts under the same conditions as described above but no radioactivity could be detected in any of the fatty acid or \(\beta\)-carotene fractions (data not shown). However, the problem of bypasses in principal is, as yet, not solved.

The following model is proposed (Fig. 4). A high affinity of the enzymes involved in carotene synthesis plus their compartmentation, channels plastid metabolites leading to a flow of substrates in the plastids toward carotene synthesis.

The model shows the substrate flow from primary photosynthetic products to amino acids, isoprenoids, and fatty acids in spinach chloroplasts. After CO\(_2\)-fixation and formation of triosephosphates most of these intermediates are exported by the phosphate translocator and used predominantly in sucrose synthesis (27). A smaller part of the C\(_3\)-precursors is converted to PEP by the plastidic phosphoglycerate mutase and enolase (15, 17, 19, 23). The literature cited therein) and dephosphorylated to pyruvate by the plastidic pyruvate kinase. PEP and E-4-P are needed for the aromatic amino acid synthesis (4). Pyruvate serves as substrate for the formation of branched chain amino acids in chloroplasts (22) and also is involved in plastidic isoprenoid formation. Fatty acid synthesis is predominantly supplied from an external site (28). Only when the plastidic isoprenoid synthesizing system is saturated by intermediate substrates (Fig. 2), does the remaining acetyl-CoA—formed by the pyruvate dehydrogenase complex (30)—supply fatty acid synthesis. This is consistent with experiments showing higher incorporation into fatty acids of labeled acetate than from NaH\(^{14}\)CO\(_3\) (19) and isotopic dilution experiments using unlabeled acetate (Fig. 3b).

A direct substrate flow from photosynthetically fixed CO\(_2\) to acetyl-CoA probably is a bypass for fatty acid synthesis, but an important pathway to provide plastid isoprenoids with substrates in spinach chloroplasts. Further investigations are needed to determine whether kinetic parameters (\(V_{\text{max}}\), \(K_{\text{m}}\)), structural organization of the relevant enzymes, compartmentation, or all contribute to this effective substrate flow.

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