Plant Physiol. (1995) 108: 609–614

Enzymatic Evidence for a Complete Oxidative Pentose Phosphate Pathway in Chloroplasts and an Incomplete Pathway in the Cytosol of Spinach Leaves[1]

Claus Schnarrenberger*, Anke Flechner, and William Martin

Institut für Pflanzenphysiologie und Mikrobiologie, Freie Universität Berlin, Königin-Luise-Strasse 12–16a, D-14195 Berlin, Germany (C.S., A.F.); and Institut für Genetik, Technische Universität Braunschweig, Spielmannstrasse 7, D-38106 Braunschweig, Germany (W.M.)

The intracellular localization of transaldolase, transketolase, ribose-5-phosphate isomerase, and ribulose-5-phosphate epimerase was reexamined in spinach (*Spinacia oleracea* L.) leaves. We found a highly predominant if not exclusive localization of these enzyme activities in chloroplasts isolated by isopyknic centrifugation in sucrose gradients. Glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glucose phosphate isomerase, and triose phosphate isomerase activity was present in the chloroplast fraction but showed additional activity in the cytosol (supernatant) fraction attributable to the cytosol-specific isoforms known to exist for these enzymes. Anion-exchange chromatography of proteins of crude extracts on diethylaminoethyl-Fractogel revealed only a single enzyme each for transaldolase, transketolase, ribose-5-phosphate isomerase, and ribulose-5-phosphate epimerase. The data indicate that chloroplasts of spinach leaf cells possess the complete complement of enzymes of the oxidative pentose phosphate pathway (OPPP), whereas the cytosol contains only the first two reactions, contrary to the widely held view that plants generally possess a cytosolic OPPP capable of cyclic function. The chloroplast enzymes transketolase, ribose-5-phosphate isomerase, and ribulose-5-phosphate epimerase appear to be amphibolic for the Calvin cycle and OPPP.

In plants, the OPPP is connected inter alia with fatty acid biosynthesis, nitrate reduction, and the shikimate pathway (Butt and Beevers, 1961; Dalling et al., 1972; Jensen, 1985). The pathway is known to occur in chloroplasts, as demonstrated by CO_2 liberation from [1-^{14}C]Glc over that of [2-^{14}C]Glc in isolated chloroplasts (Stitt and ap Rees, 1980). Yet curiously, the additional presence of a separate, complete OPPP in the cytosol is generally assumed (even in many textbooks), although evidence to support that view is largely circumstantial.

Through cell fractionation, G6PDH and 6PGDH activities were detected in both chloroplasts and cytosol (Garnier-Dardart, 1965; Heber et al., 1967; Slack and Hatch, 1967; Latzko and Gibbs, 1968; Mukerji and Ting, 1968; Miflin and Beevers, 1974). When it was shown that these two reactions were catalyzed by distinct OPPP isoenzymes in the chloroplast and cytosol, respectively (Schnarrenberger et al., 1973; Simcox and Dennis, 1978; Nishimura and Beevers, 1981), it was widely assumed thereafter, yet never clearly demonstrated, that all other activities of the pathway also occur as such isoenzymes and are compartmentalized in the same manner.

Although many cell fractionation studies (Heber et al., 1967; Emes and Fowler, 1978; Feierabend and Gringel, 1983) indicated that most of the activities in the OPPP core segment may be confined to plastids, one notable report assumed that about 70% of these activities were cytosolic (Latzko and Gibbs, 1968) in spinach leaves. Attempts to distinguish plastid- and cytosol-specific isoenzymes of Rib-5-P isomerase and transketolase from various tissues (Rutner, 1970; Kawashima and Tanabe, 1976; Feierabend and Gringel, 1983) were mostly unsuccessful, and it was recognized (Feierabend and Gringel, 1983) that the cyclic mode of the cytosolic OPPP would be problematic in the absence of corresponding isoenzymes. Little attention was given to the matter thereafter.

We reinvestigated the localization in spinach leaves of activities for all enzymes of the OPPP, i.e. G6PDH (EC 1.1.1.49), 6PGDH (EC 1.1.1.44), transketolase (EC 2.2.1.1), transaldolase (EC 2.2.1.2), Rib-5-P isomerase (EC 5.3.1.6), and ribulose-5-P 3-epimerase (EC 5.1.3.1), by cell fractionation and by anion-exchange chromatography in search of cell-compartment-specific isoenzymes. All OPPP activities were detected in chloroplasts. Yet, except for the two dehydrogenases, we could find no evidence of cytosolic OPPP isoenzymes in this tissue.

MATERIALS AND METHODS

Material

Spinach (*Spinacia oleracea* L.) leaves were collected from glasshouse plants or used after storage at −20°C.

Separation of Isoenzymes

Deribbed spinach leaves (20 g) were homogenized in 40 mL of phosphate buffer (10 mM potassium phosphate, pH

[1] This work was supported by the Deutsche Forschungsgemeinschaft (Ma 1426/3–1, Schn 28/21–1).

* Corresponding author; fax 49–30–838–4313.

Abbreviations: FBP, Fru-1,6-bisP; G6PDH, Glc-6-P dehydrogenase; OPPP, oxidative pentose phosphate pathway; 6PGDH, 6-phosphogluconate dehydrogenase.

6.5, and 10 mM 2-mercaptoethanol). The slurry was squeezed through two layers of cheesecloth and centrifuged for 40 min at 48,000*g* and at 4°C. After the pH was adjusted to 6.5, the solution was diluted with 10 mM 2-mercaptoethanol to a conductivity of less than 3 millisiemens cm^{-1} and applied to a DEAE-Fractogel (Merck, Darmstadt, Germany) column (2.5 × 10 cm) equilibrated with a buffer of pH 6.5. Proteins were eluted with a 200-mL gradient of 0 to 0.3 M KCl in phosphate buffer and collected in fractions of 5.0 mL.

Cell Fractionation

Cell organelles were separated by isopycnic centrifugation as described by Schnarrenberger et al. (1971) with some modifications. Deribbed spinach leaves (100 g) were gently homogenized in 200 mL of 0.4 M (w/w) Suc, 20 mM glycylglycine, pH 7.5, and 1 mM DTE with a mortar and pestle. This preparation was squeezed through two layers of cheesecloth and layered on top of a Suc gradient from 22.5 to 60% Suc. The gradient was generated by layering 2.5-mL aliquots of solutions of 60, 57.5, 55, 52.5, 50, 47.5, 45, 42.5, 40, 37.5, 35, 32.5, 30, 27.5, 25, 22.5% Suc solutions in 20 mM glycylglycine, pH 7.5, and 1 mM DTE. The organelle preparation was centrifuged for 3.5 h in an SW 25.2 rotor of a DuPont Combi Plus ultracentrifuge at 48,000*g*. Fractions of 1.5 mL were taken from the bottom and assayed for enzyme activities, Chl, and Suc concentration.

Enzyme Assays

Enzymes were measured photometrically by following the A_{334} change of NADH in 1-mL cuvettes at a room temperature of 20°C. Transaldolase was tested according to a procedure modified from that of Tsolas and Joris (1975) in 70 mM triethanolamine, pH 7.6, 7 mM EDTA, 3.5 mM $MgCl_2$, 1 unit each of triose-P isomerase and glycerol-3-P dehydrogenase, 240 μM NADH, 200 μM erythrose-4-P, and 3 mM Fru-6-P. Ribulose-5-P epimerase was assayed in 50 mM Tris-HCl, pH 7.5, 4.5 mM $MgCl_2$, 500 μM thiamine PPi, 0.5 unit of transketolase, 1 unit each of triose-P isomerase and glycerol-3-P dehydrogenase, 240 μM NADH, 200 μM erythrose-4-P, and 100 μM ribulose-5-P. Rib-5-P isomerase was assayed according to an assay modified from that of Tabachnick et al. (1958) in 40 mM potassium phosphate, pH 7.4, 5 mM $MgCl_2$, 0.5 unit of ribulose-5-P epimerase, 1 unit each of triose-P isomerase and glycerol-3-P dehydrogenase, 240 μM NADH, and 1 mM Rib-5-P. Transketolase was assayed according to a modified version of that used by Murphy and Walker (1982) in 10 mM Tris-HCl, pH 7.5, 4 mM $MgCl_2$, 100 μM thiamine PPi, 1 unit each of triose-P isomerase and glycerol-3-P dehydrogenase, 240 μM NADH, and substrate (5 mL of 50 mM Rib-5-P were incubated for 1 h at 37°C with 5 units each of ribulose-5-P epimerase and Rib-5-P isomerase and kept at −20°C). Aldolase, malate dehydrogenase, hydroxypyruvate reductase (Krüger and Schnarrenberger, 1983), G6PDH, 6PGDH (Schnarrenberger et al., 1973), triose-P isomerase (Beisenherz, 1955), Rubisco (Lilley and Walker, 1974), and Chl (Arnon, 1949) were tested according to published procedures. Suc concentration was determined by refractometry.

RESULTS

When proteins from a crude extract of spinach leaves were separated by anion-exchange chromatography on DEAE-Fractogel (Fig. 1), only one peak of activity was detected for transaldolase, transketolase, Rib-5-P isomerase, and ribulose-5-P epimerase. The same results were obtained when the chromatography was performed at pH 7.5 or 8.2 (data not shown). The failure to detect chloroplast/cytosol isoenzymes could be due to the mere absence of such isozymes or to the inability of the method to separate them. However, it has been possible by using anion-exchange chromatography to separate all other chloroplast/cytosol isoenzymes of sugar phosphate metabolism investigated to date, e.g. starch phosphorylase (Steup et al., 1980; Mateyka and Schnarrenberger, 1984), phosphoglucomutase (Mühlbach and Schnarrenberger, 1978), Glc-P isomerase (Schnarrenberger and Oeser, 1974), FBPase (Latzko et al., 1974), FBP aldolase (Krüger and Schnarrenberger, 1983; Lebherz et al., 1984; Schnarrenberger and Krüger, 1986), hexokinase (Schnarrenberger, 1990), G6PDH and 6PGDH (Schnarrenberger et al., 1973, 1975; Simcox et al., 1977; Simcox and Dennis, 1978; Nishimura and Beevers, 1981), glyceraldehyde-3-P dehydrogenase (Miernyk and Dennis, 1982), 3-phosphoglycerate kinase (Miernyk and Dennis, 1982; Köpke-Secundo et al., 1990; Morrow and Bradbeer, 1990), triose-P isomerase (Kurzok and Feierabend, 1984), pyruvate kinase (Ireland et al., 1979), and enolase and phosphoglycerate mutase (Miernyk and Dennis, 1982; Plaxton, 1989). It is conceivable, yet we consider it highly unlikely, that isoenzymes for transaldolase, transketolase, Rib-5-P isomerase, and ribulose-5-P epimerase exist but are undetectable. By far the most straightforward interpretation is that only a single enzyme exists for each in spinach leaves.

Cell organelle fractionation in Suc gradients by isopycnic centrifugation (Fig. 2) resolved peroxisomes (hydroxypyruvate reductase), chloroplasts (Rubisco), mitochondria (particulate peak of malate dehydrogenase), thylakoids (main peak of Chl), and a supernatant fraction (soluble or solubilized proteins). The ratio of the chloroplast marker (Rubisco) activity in the chloroplast and supernatant fractions was 1.04, providing an internal standard for comparison of chloroplast localization. Ratios of chloroplast to supernatant activity for transaldolase, transketolase, Rib-5-P isomerase, and ribulose-5-P epimerase were found to be very close to the value of 1.04 determined for Rubisco. The respective ratios were 0.84, 0.92, 0.96, and 0.95 (Table I), indicating that approximately 90% or greater of the total activity of each was localized in chloroplasts.

In contrast, the ratio of particulate and supernatant activities of G6PDH and 6PGDH were 0.05 and 0.32, respectively, indicating that about 5 and 30% of the activities were chloroplastic. The 5% chloroplast G6PDH activity detected appears to be much too low when compared to a previous study (Schnarrenberger et al., 1973), since other chloroplast activities (aldolase, Glc-P isomerase, and tri-

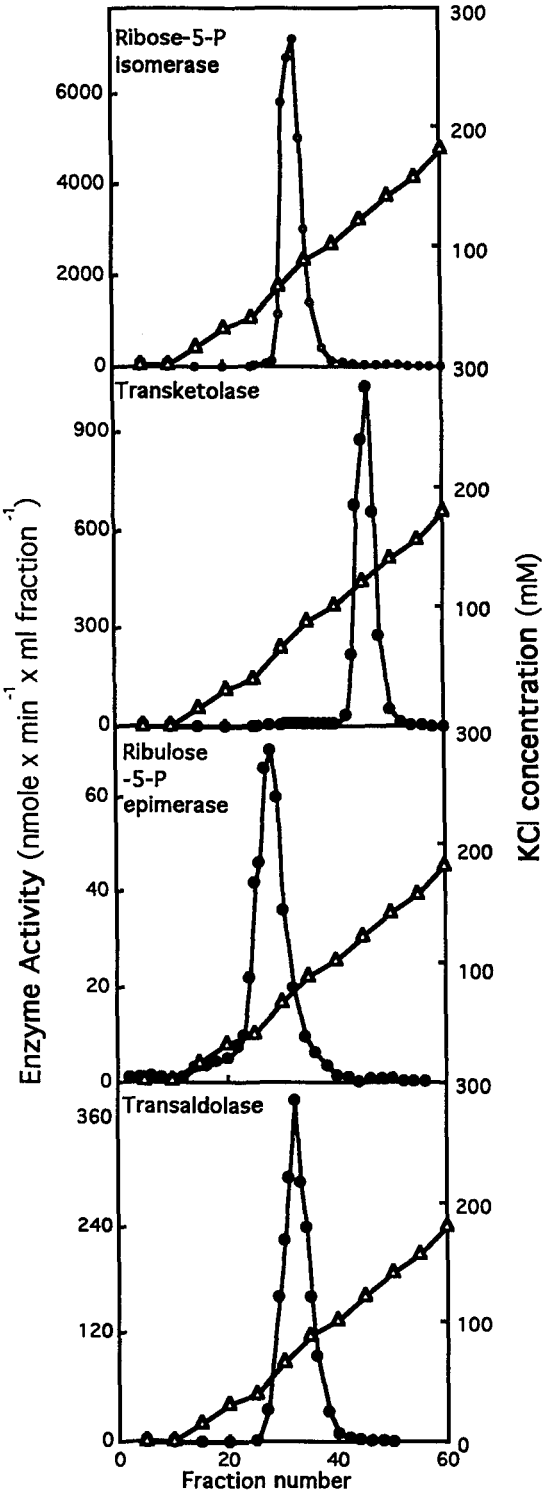

Figure 1. Enzyme profiles for Rib-5-P isomerase, transketolase, ribulose-5-P epimerase, and transaldolase after separation of proteins from a crude homogenate of spinach leaves by anion-exchange chromatography on DEAE-Fractogel.

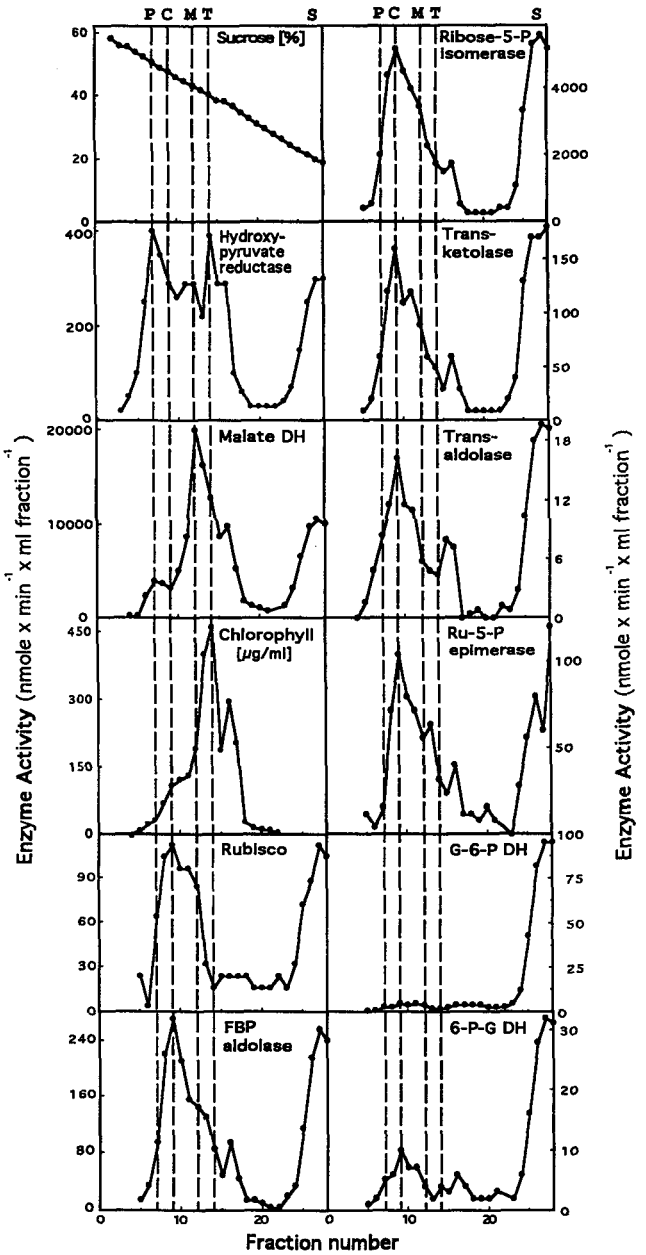

Figure 2. Suc concentration, marker enzyme activities, and enzyme activities of sugar phosphate metabolism in a Suc gradient after separation of cell organelles by isopycnic centrifugation. The peak fraction for peroxisomes (P), chloroplasts (C), mitochondria (M), and thylakoids (T) are marked by dashed lines. The three supernatant fractions (S) are about 6 times larger than the other gradient fractions. DH, Dehydrogenase.

ose-P isomerase) were recovered in quantities consistent with their isoenzyme proportions from earlier studies (Schnarrenberger and Oeser, 1974; Schnarrenberger et al., 1983; Kurzok and Feierabend, 1984; Schnarrenberger and Krüger, 1986). Chloroplast G6PDH activity is, for unknown reasons, often obscured (Simcox et al., 1977; Simcox and Dennis, 1978; Nishimura and Beevers, 1981), probably because of rapid activity loss. The enzyme is known to be

Table I. *Ratio of enzyme activities in the chloroplast fraction to the activities in the supernatant fraction (average of the activity in the top two fractions in Fig. 2) after cell organelle separation by isopycnic centrifugation*

Enzyme Activity	Ratio
Rubisco	1.04
Transaldolase	0.84
Transketolase	0.92
Rib-5-P isomerase	0.96
Ribulose-5-P epimerase	0.95
G6PHD-6-P	0.05
6PGDH	0.32
FBP aldolase	1.09
Glc-P isomerase	0.14
Triose-P isomerase	0.39

regulated by several factors, including light, reducing compounds, NADP/NADPH ratio, $MgCl_2$, and ribulose-1,5-bisP (Lendzian and Ziegler, 1970; Lendzian, 1980; Scheibe et al., 1989). In this study, inclusion of 500 μM NADP in all solutions for organelle preparation did not increase the low recovery of G6PDH activity in the chloroplast fraction (data not shown). However, the addition of 1 mM each $MgCl_2$, KCl, and EDTA instead of 1 mM DTE increased the particulate G6PDH activity 2-fold.

The chloroplast/cytosol ratios for FBP aldolase, Glc-P isomerase, and triose-P isomerase were 1.09, 0.14, and 0.38, respectively. Within the resolution of the method, this is in agreement with previous findings that the proportion of chloroplastic enzyme activity is 90, 20, and 40%, respectively, of the total activity for these enzymes in green leaves (Schnarrenberger and Oeser, 1974; Kurzok and Feierabend, 1984; Schnarrenberger, 1984; Schnarrenberger and Krüger, 1986). These results strengthen the conclusion that only little, if any, of the activities of transaldolase, transketolase, Rib-5-P isomerase, and ribulose-5-P epimerase is attributable to the cytosolic cell fraction. Thus, it appears very likely that the OPPP does not occur at all in the cytosol of this tissue or only at such low activity levels as to preclude detection with current methods.

DISCUSSION

In plant cells the first two enzymes in the OPPP, G6PDH and 6PGDH, have been more thoroughly studied than those in the regenerative segment. It was unequivocally shown that chloroplast/cytosol isoenzymes exist for G6PDH and 6PGDH both in green leaf cells (Schnarrenberger et al., 1973) and in nongreen tissues (Schnarrenberger et al., 1975; Simcox et al., 1977; Simcox and Dennis, 1978; Nishimura and Beevers, 1981). Cytosolic G6PDH has even been cloned from potato tuber (Graeve et al., 1994).

However, conflicting reports exist in the literature about the compartmentation of enzymes in the regenerative segment of the OPPP. In an early organelle fractionation study of spinach leaves (Latzko and Gibbs, 1968), 70% of the total activity of the enzymes was calculated to be cytosolic. Neither we nor other groups could find similarly high cytosolic activities. Instead, these activities were found to be more than 90% plastid localized (Heber et al., 1967; Simcox et al., 1977; Emes and Fowler, 1978; Feierabend and Gringel, 1983; this paper). Such high values already approach the resolution limit of the cell-fractionation technique, which cannot distinguish between complete and predominant plastid localization. In other experiments using anion-exchange chromatography and native gel electrophoresis, no evidence for isoenzymes of Rib-5-P isomerase (Rutner, 1970) or transketolase (Feierabend and Gringel, 1983) could be obtained in spinach, pea, or rye leaves that were congruent with our findings.

Although cytosolic isoforms for activities from the pathway's regenerative segment have been proposed to exist, the evidence for cytosolic isoenyzmes is still disputable until further reconfirmation is achieved (e.g. Rib-5-P isomerase from a chloroplast and cytosol fraction of pea shoots was reported to have a slightly different pI value [Anderson and Advani, 1970; Anderson, 1971]). However, the very small difference in pI during IEF, problems with producing pure cell fractions, and the virtually identical properties of the enzyme activities in the two cell fractions only circumstantially support the presence of a cytosolic form. In other studies, no evidence for isoenzymes of Rib-5-P isomerase in spinach and tobacco leaves could be obtained using several chromatographic procedures and native electrophoresis (Rutner, 1970; Kawashima and Tanabe, 1976).

Similarly, a slight activation of transketolase by $MgCl_2$ in the chloroplast relative to the cytosol fraction from wheat leaves was reported, but pH dependence and substrate specificity for various sugars were almost indistinguishable (Murphy and Walker, 1982). Subsequent and detailed studies of pea and rye leaf transketolase (Feierabend and Gringel, 1983) provided no evidence for isoenzymes through electrofocusing or various chromatographic techniques.

Results of the present study confirm the findings of Rutner (1970) and Feierabend and Gringel (1983) and extend their observations to the activities of transaldolase and ribulose-5-P epimerase, leading to the conclusion that the cytosol of spinach leaves is devoid of the OPPP core or contains insufficient activities to be of similar relevance to energy metabolism as the chloroplast pathway. From this it follows that a set of three activities (transketolase, Rib-5-P isomerase, and ribulose-5-P epimerase) is drawn upon by both the oxidative and the reductive pentose-P pathways in chloroplasts of this tissue, whereas transaldolase functions in the OPPP only. In another instance, a widely held view was also contradicted in that plant cells, in general, possess a full complement of plastidic and cytosolic isoenzymes of sugar phosphate metabolism. In this case *Chlamydomonas reinhardtii* and *Dunaliella bioculata* appear to lack cytosolic isoforms of glycolytic and gluconeogenetic enzymes (Klein, 1986; Schnarrenberger et al., 1990, 1994). Therefore, the a priori assumption that cytosolic isoenzymes generally exist for activities of sugar phosphate metabolism in plants should be viewed more critically until the presence of such isoenzymes is demonstrated beyond doubt.

In tissues or developmental stages with metabolic needs differing from those of spinach leaves (i.e. developing

seeds and germinating seedlings), cytosolic OPPP core isoenzymes may exist. However, the data concerning developing castor bean endosperm and pea roots tend to rather support a predominant if not exclusive plastidic localization as well as substantial cytosolic activities. In any case, if cytosolic isoenzymes really exist, which cannot be fully excluded from the discussion, a more thorough distinction of cytosol/plastid isoenzymes would be required than that presented in the past. In case of their absence, however, the question arises as to the fate of the product of the cytosolic G6PDH and 6PGDH, ribulose-5-P. Previous findings by Bassham et al. (1967) that the chloroplast envelope is easily permeable for pentose-Ps will warrant additional work to clarify the fate of this cytosolic product.

Uncertainty regarding chloroplast/cytosol isoenzymes similar to that for the OPPP has persisted for the shikimate pathway, which branches at erythrose-4-P (Jensen et al., 1989). Distinct isoenzymes of several activities in the shikimate and derivative pathways have been studied in detail, and in each case, both forms were chloroplast specific (Nyogi and Fink, 1992; Eberhard et al., 1993; Görlach et al., 1993a, 1993b; Last, 1993; Bohlmann et al., 1995; Herrmann, 1995). Up to 60% of dry weight in some tissues can consist of compounds derived from the shikimate pathway (Jensen, 1985), but the Calvin cycle cannot export large amounts of erythrose-4-P without rapidly depleting itself (Geiger and Servaites, 1994). By contrast, the OPPP can freely operate in a $2C_6 \rightarrow 2CO_2 + C_6 + C_4$ mode, making it a likely candidate source for erythrose-4-P destined for shikimate metabolism.

ACKNOWLEDGMENTS

The authors thank Mrs. Renate Grübnau for excellent technical assistance and Dr. Jörg Bohlmann and Katrin Henze for stimulating discussions.

Received December 22, 1994; accepted February 23, 1995.

LITERATURE CITED

Anderson LE (1971) Chloroplast and cytoplasmic enzymes. III. Pea leaf ribose 5-phosphate isomerases. Biochim Biophys Acta **235:** 245–249

Anderson LE, Advani VR (1970) Chloroplast and cytoplasmic enzymes. Three distinct isoenzymes associated with the reductive pentose phosphate cycle. Plant Physiol **45:** 583–585

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol **24:** 1–15

Bassham JA, Kirk M, Jensen RG (1967) Photosynthesis by isolated chloroplasts. I. Diffusion of labeled photosynthetic intermediates between isolated chloroplasts and suspending medium. Biochim Biophys Acta **153:** 211–218

Beisenherz G (1955) Triosephosphate isomerase from calf muscle. Methods Enzymol **1:** 387–391

Bohlmann J, deLuca V, Eilert U, Martin W (1995) Purification and cDNA cloning of anthranilate synthase from *Ruta graveolens*: modes of expression and properties of native and recombinant enzymes. Plant J **7:** 491–501

Butt VS, Beevers H (1961) The regulation of pathways of glucose catabolism in maize roots. Biochem J **80:** 21–27

Dalling MJ, Tolbert NE, Hageman RH (1972) Intracellular location of nitrate reductase and nitrite reductase. II. Wheat roots. Biochim Biophys Acta **283:** 513–519

Eberhard J, Raeschke H-R, Schmid J, Amrhein N (1993) Cloning and expression of a higher plant chorismate mutase. FEBS Lett **334:** 233–236

Emes MJ, Fowler MW (1978) Location of transketolase and transaldolase in apical cells of pea roots. Biochem Soc Trans **6:** 203–205

Feierabend J, Gringel G (1983) Plant transketolase: subcellular distribution, search for multiple forms, site of synthesis. Z Pflanzenphysiol **110:** 247–258

Garnier-Dardart J (1965) Activités enzymatiques des chloroplastes isolés de *Bryophyllum daigremontianum* Berger: oxydation des hexoses, formation et dégradation d'acide malique. Physiol Veg **3:** 215–227

Geiger DR, Servaites JC (1994) Diurnal regulation of photosynthetic carbon metabolism in C3 plants. Annu Rev Plant Physiol Plant Mol Biol **45:** 235–256

Görlach J, Beck A, Hemstrand JM, Handa A, Hermann K, Schmid J, Amrhein N (1993a) Differential expression of tomato (*Lycopersicon esculentum* L.) genes encoding shikimate pathway isoenzymes. I. 3-Deoxy-D-*arabino*-heptulosonate 7-phosphate synthase. Plant Mol Biol **23:** 697–706

Görlach J, Schmid J, Amrhein N (1993b) Differential expression of tomato (*Lycopersicon esculentum* L.) genes encoding shikimate pathway isoenzymes. II. Chorismate synthase. Plant Mol Biol **23:** 707–716

Graeve K, von Schaewen A, Scheibe R (1994) Purification, characterization, and cDNA sequence of glucose-6-phosphate dehydrogenase from potato (*Solanum tuberosum* L.). Plant J **5:** 353–361

Heber U, Hallier UW, Hudson MA (1967) Untersuchungen zur intrazellulären Verteilung von Enzymen und Substraten in der Blattzelle. II. Lokalisation von Enzymen des reduktiven und dem oxidativen Pentosephosphat-Zyklus in den Chloroplasten und Permeabilität der Chloroplasten-Membran gegenüber Metaboliten. Z Naturforsch **22b:** 1200–1215

Herrmann K (1995) The shikimate pathway as an entry to aromatic metabolism. Plant Physiol **107:** 7–12

Ireland RJ, DeLuca V, Dennis DT (1979) Isoenzymes of pyruvate kinase in etioplasts and chloroplasts. Plant Physiol **63:** 903–907

Jensen RA (1985) The shikimate/arogenate pathway: Link between carbohydrate metabolism and secondary metabolism. Physiol Plant **66:** 164–168

Jensen RA, Morris P, Bonner C, Zamir LO (1989) Biochemical interface between aromatic amino acid biosynthesis and secondary metabolism. *In* NG Lewis, MG Paice, eds, Plant Cell Wall Polymers: Biogenesis and Biodegradation, Symposium Series, Vol 379. American Chemical Society, Washington, DC, pp 89–107

Kawashima N, Tanabe Y (1976) Purification and properties of ribose phosphate isomerase from tobacco leaves. Plant Cell Physiol **17:** 757–764

Klein U (1986) Compartmentation of glycolysis and of oxidative pentose pathway in *Chlamydomonas reinhardtii*. Planta **167:** 81–86

Köpke-Secundo E, Molnar I, Schnarrenberger C (1990) Isolation and characterization of the cytosolic and chloroplastic 3-phosphoglycerate kinase from spinach leaves. Plant Physiol **93:** 40–47

Krüger I, Schnarrenberger C (1983) Purification, subunit structure and immunological comparison of fructose-bisphosphate aldolases from spinach and corn leaves. Eur J Biochem **136:** 101–106

Kurzok H-G, Feierabend J (1984) Comparison of a cytosolic and a chloroplast triosephosphate isomerase isoenzyme from rye leaves. I. Purification and catalytic properties. Biochim Biophys Acta **788:** 214–221

Last RL (1993) The genetics of nitrogen assimilation and amino acid biosynthesis in flowering plants: progress and prospects. Int Rev Cytol **143:** 297–330

Latzko E, Gibbs M (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

Latzko E, Zimmermann G, Feller U (1974) Evidence for a hexosediphosphatase from the cytoplasm of spinach leaves. Hoppe-Seylers Z Physiol Chem **355:** 321–326

Lebherz HG, Leadbetter MM, Bradshaw RA (1984) Isolation and characterization of the cytosolic and chloroplast form of spinach leaf fructose diphosphate aldolase. J Biol Chem **259:** 1011–1017

Lendzian KJ (1980) Modulation of glucose-6-phosphate dehydrogenase by NADPH, $NADP^+$ and dithiothreitol at variable $NADPH/NADP^+$ ratios in an illuminated reconstituted spinach (*Spinacia oleracea* L.) chloroplast system. Planta **148:** 1–6

Lendzian KJ, Ziegler H (1970) Über die Regulation der Glucose-6-phosphate Dehydrogenase in Spinat Chloroplasten durch Licht. Planta **74:** 27–36

Lilley RM, Walker DA (1974) An improved spectrometric assay for ribulosebisphosphate carboxylase. Biochim Biophys Acta **358:** 226–229

Mateyka C, Schnarrenberger C (1984) Starch phosphorylase isoenzymes in mesophyll and bundle sheath cells of corn leaves. Plant Sci Lett **36:** 119–123

Miernyk JA, Dennis DT (1982) Isoenzymes of the glycolytic enzymes in endosperm from developing castor oil seeds. Plant Physiol **69:** 825–828

Miflin BJ, Beevers H (1974) Isolation of intact plastids from a range of plant tissues. Plant Physiol **53:** 870–874

Morrow EM, Bradbeer JW (1990) Separation, purification, and comparative properties of chloroplast and cytoplasmic phosphoglycerate kinase from barley leaves. Plant Physiol **93:** 374–383

Mühlbach H, Schnarrenberger C (1978) Properties and intracellular distribution of two phosphoglucomutases from spinach leaves. Planta **141:** 65–70

Mukerji SK, Ting IP (1968) Intracellular localization of CO_2 metabolism enzymes in *Cactus* phylloclades. Phytochemistry **7:** 903–911

Murphy DJ, Walker DA (1982) The properties of transketolase from photosynthetic tissue. Planta **155:** 316–320

Nishimura M, Beevers H (1981) Isoenzymes of sugar phosphate metabolism in endosperm of germinating castor beans. Plant Physiol **67:** 1255–1258

Niyogi KK, Fink GR (1992) Two anthranilate synthase genes in *Arabidopsis*: defense-related regulation of the tryptophan pathway. Plant Cell **4:** 721–733

Plaxton WC (1989) Molecular and immunological characterisation of plastid and cytosolic pyruvate kinase isoenzymes from castor-oil-plant endosperm and leaf. Eur J Biochem **181:** 443–451

Rutner AC (1970) Spinach 5-phosphoribose isomerase. Purification and properties of the enzyme. Biochemistry **9:** 178–184

Scheibe R, Geissler A, Fickenscher K (1989) Chloroplast glucose-6-phosphate dehydrogenase: K_m shift upon light modulation and reduction. Arch Biochem Biophys **274:** 290–297

Schnarrenberger C (1990) Characterization and compartmentation, in green leaves, of hexokinases with different specificities for glucose, fructose, and mannose and for nucleoside triphosphates. Planta **181:** 249–255

Schnarrenberger C, Herbert M, Krüger I (1983) Intracellular compartmentation of isozymes of sugar phosphate metabolism in green leaves. *In* MC Rattazzi, JG Scandalios, GS Whitt, eds, Isozymes: Current Topics in Biological and Medical Research, Vol 8. Alan R. Liss, New York, pp 23–51

Schnarrenberger C, Jacobshagen S, Müller B, Krüger I (1990) Evolution of isozymes of sugar phosphate metabolism in green algae. *In* S Ogita, JS Scandalios, eds, Isozymes: Structure, Function, and Use in Biology and Medicine. Wiley-Liss, New York, pp 743–764

Schnarrenberger C, Krüger I (1986) Distinction between cytosol and chloroplast fructose-bisphosphate aldolases from pea, wheat, and corn leaves. Plant Physiol **80:** 301–304

Schnarrenberger C, Oeser A (1974) Two isoenzymes of glucose-phosphate isomerase from spinach leaves and their intracellular compartmentation. Eur J Biochem **45:** 77–82

Schnarrenberger C, Oeser A, Tolbert NE (1971) Development of microbodies in sunflower cotyledons and castor bean endosperm during germination. Plant Physiol **48:** 566–574

Schnarrenberger C, Oeser A, Tolbert NE (1973) Two isoenzymes each of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in spinach leaves. Arch Biochem Biophys **154:** 438–448

Schnarrenberger C, Pelzer-Reith B, Yatsuki H, Freund S, Jacobshagen S, Hori K (1994) Expression of the only detectable aldolase in *Chlamydomonas reinhardtii*. Arch Biochem Biophys **313:** 173–178

Schnarrenberger C, Tetour M, Herbert M (1975) Development and intracellular distribution of enzymes of the oxidative pentose phosphate cycle in radish cotyledons. Plant Physiol **56:** 836–840

Simcox PD, Dennis DT (1978) Isoenzymes of the glycolytic and pentose phosphate pathways in proplastids from the developing endosperm of *Ricinus communis* L. Plant Physiol **61:** 871–877

Simcox PD, Reid EE, Canvin DT, Dennis DT (1977) Enzymes of the glycolytic and pentose phosphate pathways in plastids from the developing endosperm of *Ricinus communis* L. Plant Physiol **59:** 1128–1132

Slack CR, Hatch MD (1967) Comparative studies on the activity of carboxylases and other enzymes in relation to the new pathway of photosynthetic carbon dioxide fixation in tropical grasses. Biochem J **103:** 660–665

Steup M, Schächtele C, Latzko E (1980) Separation and partial characterization of chloroplast and non-chloroplast α-glucan phosphorylases from spinach leaves. Z Pflanzenphysiol **96:** 365–374

Stitt M, ap Rees T (1980) Estimation of the activity of the oxidative pentose phosphate pathway in pea chloroplasts. Phytochemistry **19:** 1583–1585

Tabachnick M, Srere PA, Cooper J, Racker E (1958) The oxidative pentose phosphate cycle. III. The interconversion of ribose 5-phosphate, ribulose 5-phosphate and xylulose 5-phosphate. Arch Biochem Biophys **74:** 315–325

Tsolas O, Joris L (1975) Transaldolase. Methods Enzymol **42:** 290–297