Species-Specific Differences in Acetyl Coenzyme A Synthesis of Chloroplasts

Hans-Jürgen Treede, Burgi Riens, and Klaus-Peter Heise

Institut für Biochemie der Pflanze der Universität Göttingen, Untere Karspüle 2, D-3400 Göttingen, Bundesrepublik Deutschland

Z. Naturforsch. **41c**, 733–740 (1986); received March 21/April 17, 1986

Acetyl-CoA and Fatty Acid Synthesis, Chloroplasts

The present investigation indicates that photosynthetically active chloroplasts can synthesize acetyl-CoA either from acetate *via* acetyl-CoA synthetase (ACS) or from pyruvate *via* the pyruvate dehydrogenase complex (PDC). Both enzyme systems have been assayed in rapidly prepared extracts of chloroplasts isolated from spinach, peas and maize mesophyll. Their kinetic properties showed few species-specific differences. The differing pyruvate and acetate concentrations within the corresponding leaf tissues have been interpreted, therefore, as constituting a major factor determining the relative involvement of both acetyl-CoA synthesizing systems within the different types of chloroplasts. The idea that acetate originates from mitochondria and pyruvate from the cytosol has been supported by nonaqueous fractionation studies. Diffusion-mediated faster uptake of acetate may indicate a predominant role of the ACS in spinach chloroplasts. Higher cellular pyruvate/acetate-ratios (2–5) in pea and maize leaves may enhance pyruvate uptake into chloroplasts and thus PDC-driven acetyl-CoA synthesis in pea and maize mesophyll chloroplasts. Maize mesophyll chloroplasts even show a light-driven pyruvate uptake accompanied by a stimulated acetyl-CoA and fatty acid formation. Assuming light-dependent increasing parameters in the stroma space, like Mg^{2+}-concentrations, pH and ATP, as further control criteria in chloroplast acetyl-CoA formation, the ACS appears better adapted to the circumstances in illuminated chloroplasts because of the fact that 1. the ACS requires these cofactors altogether; 2. the PDC is stimulated by increasing pH (up to 8) and Mg-levels (up to 5 mM) alone.

Introduction

Recent investigations indicate the coexistence of two pathways for acetyl-CoA synthesis in photosynthetically active chloroplasts of different species [1–5]. One operates through the action of acetyl-CoA synthetase (ACS), the other through the pyruvate dehydrogenase complex (PDC). It has been proposed that both enzymes are primarily controlled by the stromal levels of their substrates acetate and pyruvate [5]. Both metabolites seem to originate mainly from outside the chloroplast and to enter the plastid by either diffusion [6–8] or carrier mediated transport (postulated for pyruvate concentrations lower than 1 mM only) [8]. There is also evidence supporting an additional formation of pyruvate in spinach chloroplasts from 3-PGA by plastidic isoenzymes [9]. Thus, in spinach leaves, acetate may be provided by acetyl-CoA hydrolysis in mitochondria [10] while pyruvate may be predominantly synthesized in the cytosol [11, 12] and to a lesser degree within the chloroplasts [9]. Because of deficient acetyl-CoA hydrolyzing activities in pea mitochondria [13], a generalization of these species-specific findings appears questionable. However, it has been recently shown that especially in spinach chloroplasts 1. the calculated stromal metabolite levels appear to favor acetate as substrate for acetyl-CoA- and subsequent fatty acid formation [5]; 2. the acetyl-CoA synthesized from acetate (*via* ACS) seems to inhibit its formation from pyruvate [5] at the expense of branched-chain amino acids by feedback control [14].

The pathway of acetyl-CoA formation has been studied here for those chloroplasts which are not limited in pyruvate content. Stromal accumulation of pyruvate in this case in question is either due to enhanced cellular concentrations of this metabolite or by light-dependent active transport as recently shown for maize mesophyll chloroplasts [15]. These chloroplasts are suitable for *in vitro* studies of the

Abbreviations: ACS, acetyl-CoA synthetase; ACP, acyl-carrier protein; BSA, bovine serum albumin; CoA, coenzyme A; CS, citrate synthetase; DTE, dithioerythritol; GAP-DH, glyceraldehyde 3-phosphate dehydrogenase; PDC, pyruvate dehydrogenase complex; PEP-CX, phosphoenolpyruvate carboxylase; 3-PGA, 3-phosphoglycerate.

Reprint requests to Dr. K.-P. Heise.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/86/0700–0733 $ 01.30/0

two coexisting acetyl-CoA synthesizing enzymes within the chloroplast.

Materials and Methods

The cultivation of spinach [16], peas [5], and maize [15] has been described earlier. The overall metabolite levels have been determined in protein free extracts of the different leaf tissues. A known quantity (on a chlorophyll or protein base) of leaf sections (without middle rib) was homogenized in liquid N_2. The resulting fine powder was moderately acidified with 5% $HClO_4$, gradually thawed under continued homogenization, kept 15 min at 4 °C, centrifuged and neutralized with 5 M KOH-1 M Tricine modified according to Stitt *et al.* [17]. Aliquots of the eluates were analyzed for either acetate [5] or pyruvate with enzymatic kits from Boehringer Mannheim (FRG) with a double beam spectrophotometer (Sigma ZFP 22). Chl. was assayed according to Arnon [18] and protein after the method by Lowry [19]. The *in vivo-distribution of metabolites* in leaf cells has been investigated by a nonaqueous fractionation technique of dry-powders from spinach leaves prepared by thorough homogenization and subsequent lyophilization of quickly frozen leaf material according to Gerhardt *et al.* [20].

Isolation, purification and critical examination of intact spinach and pea chloroplasts was carried out as in [5, 16] and that of maize mesophyll chloroplasts as in [21]. The preparation methods of crude chloroplast extracts including the determinations of ACS and PDC activities have been described elsewhere [5, 16]. Acetyl-CoA formation by intact chloroplast suspensions has been measured by two radiochemical methods based either on the adsorption of the ^{14}C-labelled acetyl-CoA to charcoal [22] or on acetyl-CoA trapping with dithioerythritol [3]. Incubation conditions for acetyl-CoA and fatty acid synthesis in maize mesophyll chloroplasts are given in the legends to tables and figures and extraction procedures and measurement of labelled fatty acids were as described in [23].

Results

1. Overall levels of acetate and pyruvate

Before studying in detail the species-dependent capacity of photosynthetically active chloroplasts for the synthesis of acetyl-CoA from either pyruvate or acetate, we measured concentrations of both metabolites in protein-free extracts of leaf tissues. Losses during the elution of the homogenates were minimized by working at low temperatures and by using moderate acidification and neutralization according to Stitt *et al.* [17]. Aliquots of the eluates were stored in liquid N_2 prior to assay.

As shown in Table I, the observed significant differences in the endogenous acetate and pyruvate levels of the leaf tissues suggest species-specific variations in the availability of both precursors for acetyl-CoA formation. Thus, in extracts from spinach the acetate concentration (59 ± 20 nmol · mg^{-1} Chl) exceeded that of pyruvate (20 ± 10 nmol · mg^{-1} Chl) by a factor of 3. In corresponding measurements with peas and maize a 2–5-fold higher pyruvate than acetate level was determined. Pea and maize tissues contained about 3–9-fold more pyruvate but only half of the acetate level found with spinach. Pronounced changes in the intracellular acetate and pyruvate concentrations of the same species appear to take place depending on the physiological state of the leaf material. Thus, a decrease of pyruvate in leaf cells in the light and its increase after darkening has been inter-

Table I. Determination of acetate and pyruvate concentrations in protein-free extracts of different leaf tissues. Results are mean values of 5–10 experiments.

Leaf tissue	Metabolite concentrations [nmol · mg^{-1} protein]		[nmol · mg^{-1} Chl]		Overall concentrations* [μM]	
	pyruvate	acetate	pyruvate	acetate	pyruvate	acetate
Spinach	1 ± 0.5	3 ± 1	20 ± 10	59 ± 20	33 ± 16	98 ± 33
Peas	3.5 ± 1	2 ± 0.5	66 ± 19	38 ± 10	111 ± 32	63 ± 16
Maize	11 ± 2.5	2.5 ± 1	185 ± 42	39 ± 23	308 ± 70	65 ± 38

* For estimations of overall concentrations it has been assumed that 1 mg Chl is approximately equally distributed in 600 μl of cell sap.

preted in terms of an inhibition of glycolysis in the light and its reversal in the dark [24]. For estimation of the endogenous overall concentrations of both substrates it has been assumed that 1 mg of chlorophyll is approximately equally distributed in 600 µl of cell sap. This value has been determined by dehydration of either leaf sections or of their homogenates and showed little variation within the different leaf tissues investigated. Based on this assumption endogenous acetate concentrations were generally between 30 and 130 µM and between 65 and 130 µM in spinach leaves (Table I). These values agree better with those recently reported for young spinach leaves (70 µM) [2] than with the significantly higher concentrations (> 1 mM) found in earlier determinations [22]. The calculated endogenous pyruvate levels in the same leaf tissues varied between 33 ± 16 µM in spinach and 111 ± 32 µM in peas or 308 ± 70 µM in maize (Table I). On a chlorophyll basis the overall content of pyruvate in maize leaves (185 ± 42 nmol · mg^{-1} Chl) is in agreement with that recently found by Stitt *et al.* [25].

For further rough estimations of the subcellular metabolite distribution in spinach leaf cells it appears commendable to treat the leaf as a uniform metabolic compartment representing an average mesophyll cell with 200 µl · mg^{-1} Chl in space being subdivided into chloroplast (25 µl · mg^{-1} Chl), mitochondrial, cytosolic (20 µl · mg^{-1} Chl) and vacuolar compartment (150 µl · mg^{-1} Chl) [20]. From this point of view, the mesophyll as predominant cell type appears to make up one third of the total cell sap in spinach leaf tissue (600 µl · mg^{-1} Chl) only.

2. *Intracellular distribution of acetate and pyruvate*

At present there is little information concerning the cellular distribution and the stromal level of both metabolites. Thus, preliminary rough estimates of the stromal pyruvate amounts were in the order 0.1 mM in both spinach [26] and pea chloroplasts [8]. In order to get additional information on the levels of acetate and pyruvate in the chloroplast *in vivo*, we have tried to estimate their stromal concentration by a nonaqueous fractionation of lyophilized cell debris homogenized under liquid N_2 [20]. As indicated by marker enzymes, fractionation of this dry powder on a heptane/carbon tetrachloride-gradient leads to typical distribution patterns of subcellular compartments with their metabolites included and thus allows for rough estimates of the substrate levels within the chloroplast stroma (Fig. 1). After fractionation of

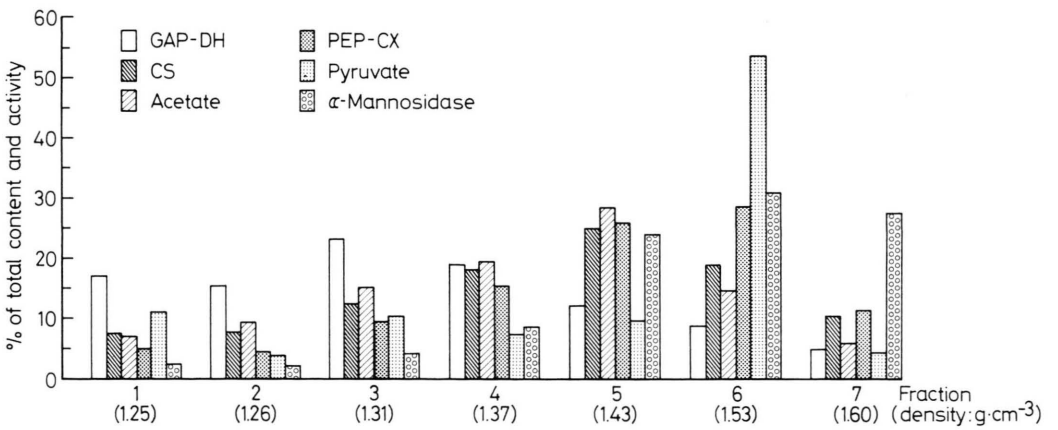

Fig. 1. An exemplary* distribution pattern of acetate and pyruvate as compared to that of marker enzymes after fractionation of finely suspended lyophilized leaf powders from spinach on a gradient of nonaqueous solvents (CCl_4/C_7H_{16}). For the preparation of the dry powder, spinach leaves were freeze stopped and the frozen tissue was ground and lyophilized. Finely dispersed suspensions of this dry material have been obtained by sonication in the same solvent mixtures.

(* Pronounced variations in the fractionation patterns of different samples, which are probably due to the heterogeneity of leaf cell populations [20] as well as to diurnal changes in the subcellular level and distribution of metabolites [24], led to the selection of one gradient representative of a couple of experiments. The presented gradient showed the best separation of the marker enzymes investigated and a good recovery of the original metabolite levels and enzyme activities before separation.)

lyophilized spinach leaf powders by this method, most of the cellular acetate (30%) coincided with the maximum of citrate synthetase (CS) activities (25%) as mitochondrial marker [27], while pyruvate (54%) was enriched in the cytosolic compartment indicated by the fractions with maximum PEP-carboxylase (PEP-CX) activities (30%) (Fig. 1). Despite the limitations of the method [20], both substrates could be recovered after fractionation with remarkable yields (acetate with approximately 100% and pyruvate with about 70% of the initial concentrations before separation). Application of this technique did not allow, however, for a distinct separation of mitochondria (with CS as marker), cytosol (with PEP-CX as indicator) and vacuoles (with α-mannosidase as marker) and thus for a more precise evaluation of the subcellular distribution of acetate and pyruvate. Nevertheless, an accumulation of acetate together with mitochondrial fragments on one hand and of pyruvate together with cytosolic particles on the other (Fig. 1) supports the postulated origin of both substrates [10, 12] at least in spinach leaf cells.

Relatively lower amounts of acetate (15–20%) and pyruvate (10%) appeared to coincide with plastid debris indicated by glyceraldehyde 3-phosphate dehydrogenase (GAP-DH) as stromal marker (Fig. 1). Comparable pyruvate values in the chloroplast compartment (7% of total extract) have been recently found from radioactive distribution patterns of metabolites after fractionation of $^{14}CO_2$-labelled wheat leaf protoplasts [28].

Based on the overall concentrations of both substrates in Table I, their percentage amount, given above, would be tantamount to 6–16 nmol · mg^{-1} Chl for acetate and to 1–3 nmol · mg^{-1} Chl for pyruvate in spinach chloroplasts. Assuming a stromal volume of 25 µl · mg^{-1} Chl [20], concentrations of about 0.2–0.6 mM for acetate and of about 40–120 µM for pyruvate can be estimated. These concentrations are similar to those taken as basis in our recent calculations [5]. The same calculation criteria provided, for the substrate levels of both acetate and pyruvate in pea chloroplasts values between 170 and 380 µM can be estimated which, in contrast to other authors [8], point to higher pyruvate concentrations than in spinach chloroplasts.

3. Substrate requirements of ACS and PDC

For a better understanding of the regulatory mechanism determining the function of the two alternative physiological pathways for acetyl-CoA synthesis [2, 3, 5] and for comparison with the calculated stromal acetate and pyruvate levels we have been interested in the kinetic properties of both enzymes in rapidly prepared chloroplast extracts. At first glance, the kinetic parameters of the stromal ACS- and PDC-activities (Table II) showed insignificant and species-specific differences only. Nevertheless, in contrast to maize mesophyll chloroplasts, the PDC of chloroplast extracts from C_3-type (especially of spinach) appeared to show as a rule higher substrate requirements (app. K_m for pyruvate = 0.1–0.6 mM) than ACS (app. K_m for acetate = 0.1–0.15 mM). The observation that higher maximal ACS- than PDC activities have generally been measured (Table II) in the chloroplast extracts does, however, not necessarily correspond to the relative reaction rates under *in vivo*-conditions, because the environmental conditions within the respective plastid compartments were not duplicated.

Table II. Kinetic measurements of the pyruvate dehydrogenase complex and acetyl-CoA synthetase in extracts from chloroplasts of different plant species. The extracts have been prepared from lyophilized or Triton X-100-lysed chloroplasts and the assays were composed as described in Materials and Methods. The data result from measurements of CoASH-dependent rates of NAD reduction.

Enzyme	Substrate	Apparent K_m [mM]			V_{max} [µmol of substrate converted · mg^{-1} Chl · h^{-1}]		
		Spinach	Peas	Maize (mesophyll)	Spinach	Peas	Maize (mesophyll)
Pyruvate dehydrogenase complex	pyruvate	0.2–0.6*	0.1–0.3	0.1–0.15	0.6–1.2	1–2	1
Acetyl-CoA synthetase	acetate	0.1	0.1–0.15	0.1–0.15	3 –4	1–2	3–4

* The higher values (> 0.3 mM) result from measurements in green extracts from Triton X-100-lysed spinach chloroplasts.

Table III. Synthesis of acetyl coenzyme A and fatty acids from either [1-^{14}C]acetate or [2-^{14}C]pyruvate by intact maize mesophyll chloroplasts *in vitro*. The incubation medium contained: 0.33 M sorbitol, 50 mM Hepes/KOH (pH 7.8), 2 mM EDTA, 1 mM ATP, 0.5 mM CoASH, 4mM $MgCl_2$, 10 mM $NaHCO_3$, 10 mM oxaloacetate, 20 mM DTE, 0.1% BSA, 100 µg Chl/ml and either 0.24 mM [1-^{14}C]acetate or [2-^{14}C]pyruvate (10 µCi/µmol). Substrate incorporation was followed for 10 and 20 min (20 °C) either in the dark or under saturating red light. The data are mean values of both incubation periods from at least 5 experiments.

	I Acetyl-CoA formation from		II Fatty acid formation from		O_2-evolution of chloroplasts
	[1-^{14}C]acetate	[2-^{14}C]pyruvate	[1-^{14}C]acetate	[2-^{14}C]pyruvate	
	[nmol · mg^{-1} Chl · h^{-1}]		[nmol · mg^{-1} Chl · h^{-1}]		[µmol · mg^{-1} Chl · h^{-1}]
dark	34	17	18	3	–
light	87	66	54	39	26

4. Synthesis of acetyl-CoA and fatty acids by maize mesophyll chloroplasts

The 5-fold excess of pyruvate as compared to acetate in maize leaf tissues (Table I), which can additionally be accumulated in the chloroplast stroma by light-dependent active transport [15] appears to favor PDC-driven acetyl-CoA formation in maize mesophyll chloroplasts *in vivo*. In order to test the above assumption, the light-dependent incorporation of [1-^{14}C]acetate and [2-^{14}C]pyruvate into acetyl-CoA and fatty acids by functionally intact (generally about 85% intactness) mesophyll chloroplasts from maize has been measured (Table III and Fig. 1). For a better comparison, both metabolites were offered uniformly diluted in specific radioactivity and pyruvate was nearly free from acetate contamination [14]. Functional integrity of the chloroplasts during the incorporation experiments was followed by simultaneous measurement of light-dependent O_2 evolution (about 30 µmol · mg^{-1} Chl · h^{-1}) in the presence of oxaloacetate, which is photoreduced *via* NADP-malate dehydrogenase in the chloroplast stroma [21, 29]. Furthermore, the suspension medium has been adjusted to optimal metabolite and cofactor conditions for acetyl-CoA [5, 16] as well as for fatty acid synthesis [30, 31] from either acetate or pyruvate (see legend to Table III). As shown in Table III, acetyl-CoA and fatty acid formation by intact maize mesophyll chloroplasts from both substrates was significantly stimulated by light. But, while in this C_4-type of chloroplasts the light-dependence of pyruvate incorporation is primarily due to a light-driven pyruvate uptake [15], that of acetate conversion appears to be mainly due to changes in the MgATP-level and only secondly to other light-dependent variable parameters in the chloroplast stroma (H^+- and Mg^{2+}-concentrations) [16, 31]. The light-dependence of both pathways is further documented by incorporation kinetics in which the net rate of the light-induced acetyl-CoA and fatty acid formation is compared with that of light/dark-transitions (Fig. 2). As shown in Fig. 2 the net rate in acetyl-CoA- (Fig. 2 B_1 and B_2) and fatty acid synthesis (Fig. 2 A_1 and A_2) from both substrates showed comparable, nearly linear increases in the light and could be interrupted by intermittent dark periods (dotted lines) or regenerated by short subsequent illumination periods of 5 min. This short-term illumination following a dark period did not suffice, however, to regenerate acetyl-CoA formation from acetate (Fig. 2 B_2) which may be due to a retarded light recovery of the low dark level of MgATP, the most important cofactor of the ACS [16]. The rapid induction by light of fatty acid synthesis from acetate after a short dark period appears, however, not to be limited by this retarded acetyl-CoA formation (Fig. 2 A_2). It may reflect the rapid new synthesis of long-chain acyl-ACPs from existing shorter precursors in the light [32].

Discussion

Taken together, the present results are further strong evidence for the coexistence in photosynthetically active chloroplasts of two acetyl-CoA synthesizing pathways, namely the ACS and the PDC [1–5]. Furthermore, the substrates of both enzymes (acetate and pyruvate) appear to originate mainly outside the chloroplast (Fig. 1) [10–12]. Therefore, the extraplastidial levels of both substrates, which have been shown to vary in the different leaf tissues inves-

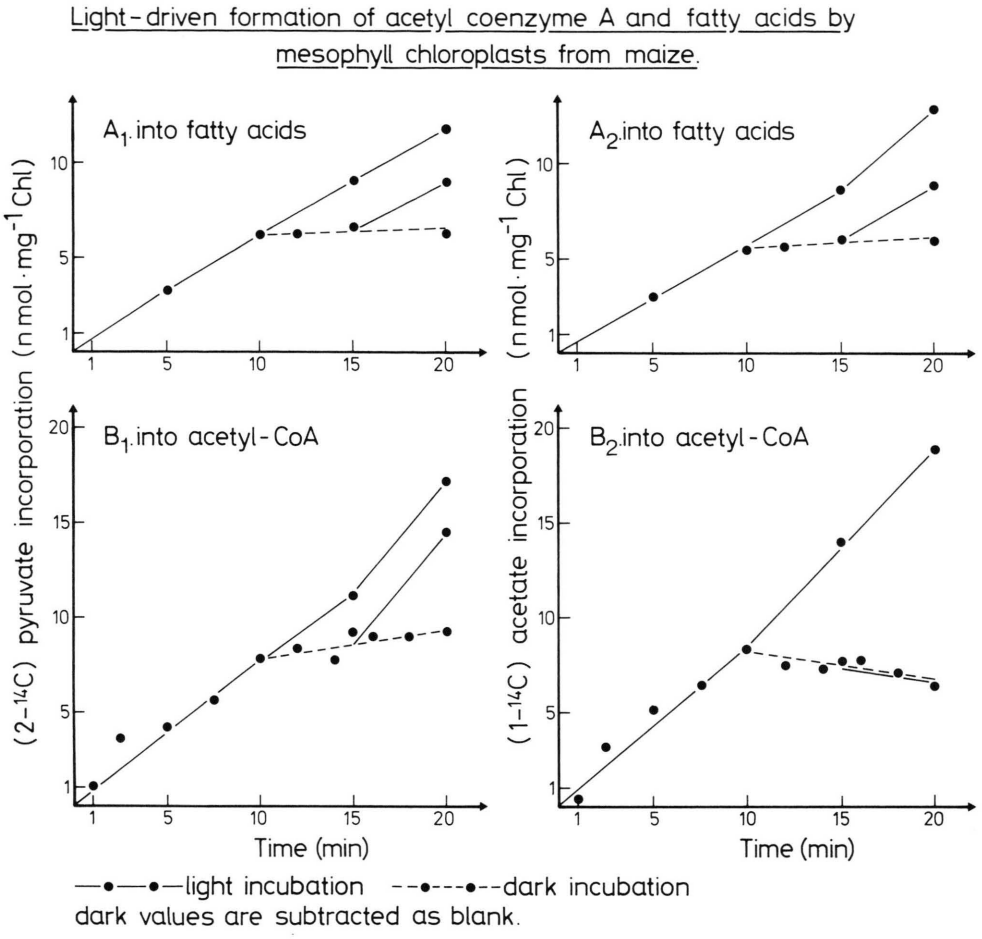

Fig. 2. Differences in the time course of the light-driven incorporation of [2-^{14}C]pyruvate and [1-^{14}C]acetate (—•—•—) by functionally intact maize mesophyll chloroplasts into either acetyl-CoA (B_1 and B_2) or fatty acids (A_1 and A_2) and its behaviour during intermittent dark periods (-•---•-). The corresponding dark values have been subtracted as blanks. The incubation conditions were as in the legend to Table III. The data are mean values of at least 3 independent experiments.

tigated (Table I), as well as the mechanism of their uptake (diffusion or carrier-mediated transport) are considered to be important factors determining the substrate availability and thus, the path of acetyl-CoA formation within the different types of chloroplasts. A controlled carrier-mediated transport into the chloroplast has been shown for exogenous pyruvate concentrations lower than 1 mM [8], while acetate [6, 7] and higher pyruvate concentrations [8] appear to penetrate the envelope by passive diffusion. Based on the endogenous acetate and pyruvate concentrations in leaf tissue (Table I) and assuming a cytosolic volume of 20 $\mu l \cdot mg^{-1}$ Chl $\cdot h^{-1}$ an extraplastidial pyruvate concentration of lower than 1 mM in combination with a controlled (carrier-mediated) pyruvate uptake seem to occur only in spinach chloroplasts. Furthermore, in view of the stromal acetate (0.2–0.6 mM) and pyruvate levels (40–120 μM) estimated above and of the substrate requirements of the ACS (app. K_m for acetate = 0.1 mM) and the PDC (app. K_m for pyruvate = 0.2–0.6 mM) shown in Table II, the ACS will be the preferred enzyme for acetyl-CoA synthesis in spinach chloroplasts [1, 2, 5]. This enzyme is further stimulated by increasing pH, MgATP- and Mg-concentrations in the stroma space under photosynthetic conditions [16, 31] while the PDC requires high Mg^{2+}-concentrations ($\geqq$ 5 mM) and an alkaline pH optimum (pH 8) alone [4, 5] and is apparently inhibited by free ATP-concentrations (*e.g.* due to Mg-complexation) [5]. Acetyl-CoA for-

mation via PDC appears further to be regulated by feedback control [5, 14].

If the estimated extraplastidial pyruvate concentrations exceed 1 mM and thus are higher than those of acetate, as for example in pea and maize leaf tissues (Table I), they may accumulate in the plastid and thus stimulate the PDC-activities in this compartment (Table III, Fig. 2) [2–5]. Thus, the increasing involvement of pyruvate as compared to acetate in acetyl-CoA formation of pea chloroplasts *in vivo* has been concluded from the following observations:

1. An acetyl-CoA hydrolase in pea mitochondria as a possible source for acetate appears to be absent [13].

2. In pea leaf tissue the endogenous pyruvate concentrations (66 ± 19 nmol · mg^{-1} Chl) exceeded that of acetate (38 ± 10 nmol · mg^{-1} Chl) (Table I) suggesting higher pyruvate levels in pea (190–340 μM) than in spinach chloroplasts (40–120 μM).

3. In rapidly prepared extracts from pea chloroplasts both acetyl-CoA synthesizing enzymes showed comparable maximal activities and substrate requirements (Table II).

Unlike C_3 species, the chloroplasts from maize mesophyll are apparently not limited in pyruvate uptake by their capacity to accumulate this substrate in the light. In isolated maize mesophyll chloroplasts [15] the V_{max} for pyruvate transport in the dark [(4.5 μmol · mg^{-1} Chl · h^{-1}) (4 °C), which resembles that in illuminated pea chloroplasts (6.5 μmol · mg^{-1} Chl · h^{-1}) (4 °C, green light) [8]] was more than 7-fold stimulated in the light (35 μmol · mg^{-1} Chl · h^{-1}) (4 °C) and even further increased at higher temperatures (95 μmol · mg^{-1} Chl · h^{-1}) (20 °C). Furthermore, the apparent affinity of the pyruvate transport towards its substrate (app. K_m = 0.8–0.9 mM) was 3-fold higher than in pea chloroplasts (app. K_m = 0.33 mM) [8], but not affected by light [15]. In contrast to C_3 chloroplasts, in which the PDC usually showed higher substrate requirements than the ACS, maize mesophyll chloroplast extracts contained both acetyl-CoA synthesizing enzymes with identical K_m-values for their substrate (0.1–0.15 mM) (Table II). Assuming, that in maize leaves *in vivo* the mesophyll tissue contains pyruvate and acetate in the same proportion as in the total leaf extract (Table I), the mesophyll chloroplasts are surrounded by a 5-fold higher pyruvate than acetate concentration. This assumption suggests, however, an accumulation of pyruvate as compared to acetate in maize mesophyll chloroplasts during *in vivo*-photosynthesis. With respect to the substrate requirements of both acetyl-CoA synthesizing enzymes (ACS and PDC) in the stroma space (Table II), pyruvate accumulation in this C_4-type of chloroplast may favor acetyl-CoA formation via the PDC except for limitations by its rapid conversion by pyruvate phosphate dikinase [29].

It is worth noting that the situation may be quite different in non photosynthetic proplastids where the PDC is very active and there is a full complement of enzymes for conversion of 3-PGA to pyruvate within the proplastid [33, 34].

The high latency of the spinach chloroplast PDC [5] is further manifested by its lability against purification procedures [35]. Thus, the multienzyme complex from spinach chloroplasts could be purified without desintegration of the holocomplex (*i.e.* loss of lipoamide dehydrogenase activities) only by using ultracentrifugation. The PDC of chloroplasts from peas [4] and maize mesophyll [35], however, could be further enriched by separation methods like: polyethylenglycol precipitation, gel chromatography and density gradient centrifugation on glycerol.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft. The authors thank Professors C. V. Givan and G. Schultz for critical discussions and Dr. H. J. Rurainski for assistance in preparing the manuscript.

[1] D. J. Murphy and P. K. Stumpf, Arch. Biochem. Biophys. **212,** 730–739 (1981).
[2] B. Liedvogel, Z. Naturforsch. **40c,** 182–188 (1985).
[3] B. Liedvogel, Anal. Biochem. **148,** 182–189 (1985).
[4] P. J. Camp and D. D. Randall, Plant Physiol. **77,** 571–577 (1985).
[5] H.-J. Treede and K.-P. Heise, Z. Naturforsch. **40c,** 496–502 (1985).
[6] B. S. Jacobson and P. K. Stumpf, Arch. Biochem. Biophys. **153,** 656–663 (1972).
[7] A. Sauer and K.-P. Heise, Z. Naturforsch. **38c,** 399–404 (1983).
[8] M. O. Proudlove and D. A. Thurman, New Phytol. **88,** 255–264 (1981).
[9] D. Schulze-Siebert, A. Heintze, and G. Schultz, submitted to Biochem. Journal.
[10] B. Liedvogel and P. K. Stumpf, Plant Physiol. **69,** 897–903 (1982).
[11] M. Stitt and T. ap Rees, Phytochemistry **18,** 1905–1911 (1979).
[12] C. V. Givan, Physiol. Plant **57,** 311–316 (1983).
[13] C. V. Givan and J. W. Hodgson, Plant Sci. Lett. **32,** 233–242 (1983).
[14] U. Homeyer, D. Schulze-Siebert, and G. Schultz, J. Plant Physiol. **119,** 87–91 (1985).
[15] U. I. Flügge, M. Stitt, and H. W. Heldt, FEBS Lett. **183,** 335–339 (1985).
[16] A. Sauer and K.-P. Heise, Z. Naturforsch. **39,** 268–275 (1984).
[17] M. Stitt and H. W. Heldt, Planta **164,** 179–188 (1985).
[18] D. J. Arnon, Plant Physiol. **24,** 1–15 (1949).
[19] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem. **193,** 265–275 (1951).
[20] R. Gerhardt and H. W. Heldt, Plant Physiol. **75,** 542–547 (1984).
[21] C. L. D. Jenkins and V. J. Russ, Plant Sci. Lett. **35,** 19–24 (1984).
[22] D. N. Kuhn, M. J. Knauf, and P. K. Stumpf, Arch. Biochem. Biophys. **209,** 441–450 (1981).
[23] A. Sauer and K.-P. Heise, Z. Naturforsch. **37c,** 218–225 (1982).
[24] U. Heber and K. A. Santarius, Biochim. Biophys. Acta **109,** 390–408 (1965).
[25] M. Stitt and H. W. Heldt, Biochim. Biophys. Acta **808,** 400–414 (1985).
[26] D. Schulze-Siebert, D. Heineke, H. Scharf, and G. Schultz, Plant Physiol. **76,** 465–471 (1984).
[27] B. A. Elias and C. V. Givan, Planta **142,** 317–320 (1978).
[28] C. Giersch, U. Heber, G. Kaiser, D. A. Walker, and S. P. Robinson, Arch. Biochem. Biophys. **205,** 246–259 (1980).
[29] M. Hatch and C. B. Osmond, in: Encyclopedia of Plant Physiology (C. R. Stocking and U. Heber, eds.), **Vol. 3,** pp. 144–184, Springer Verlag, Berlin 1976.
[30] A. Sauer and K.-P. Heise, Z. Naturforsch. **39,** 593–599 (1984).
[31] A. Sauer and K.-P. Heise, Plant Physiol. **73,** 11–15 (1983).
[32] J. Soll and P. G. Roughan, FEBS Lett. **146,** 189–192 (1982).
[33] E.-P. Journet and R. Douce, Plant Physiol. **79,** 458–467 (1985).
[34] J. A. Miernyk and D. T. Dennis, J. Exper. Botany **34,** 712–718 (1983).
[35] H. J. Treede and K.-P. Heise, in preparation.