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Distribution of Pyruvate Dehydrogenase Complex Activities between Chloroplasts and Mitochondria from Leaves of Different Species[1]

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Protoplasts from barley (*Hordeum vulgare*), pea (*Pisum sativum*), wheat (*Triticum aestivum*), and spinach (*Spinacia oleracea*) leaves were fractionated into chloroplast- and mitochondrion-enriched fractions. Pyruvate dehydrogenase complex capacities in mitochondria (mtPDC) and chloroplasts (cpPDC) were measured in appropriate fractions under conditions optimal for each isozyme. The total cellular capacity of PDC was similar in barley and pea but about 50% lower in wheat and spinach. In pea a distribution of 87% mtPDC and 13% cpPDC was found on a cellular basis. In barley, wheat, and spinach the subcellular distribution was the opposite, with about 15% mtPDC and 85% cpPDC. cpPDC activity was constant at about 0.1 nmol $cell^{-1}$ h^{-1} in cells from different regions along the developing barley leaf and showed no correlation with developmental patterns of photosynthetic parameters, such as increasing Chl and NADP-glyceraldehyde-3-phosphate dehydrogenase activity. Similarly, the capacity of the mitochondrial isoform did not change during barley leaf development and had a developmental pattern similar to that of citrate synthase and fumarase. Differences in subcellular distribution of PDCs in barley and pea are proposed to be due to differences in regulation, not to changes in isozyme proportions during leaf development or to species-specific differences in phosphorylation state of mtPDC after organelle separation.

The PDC catalyzes the conversion of pyruvate to acetyl-CoA and concomitantly reduces NAD to NADH. Plant cells are unique in that the complex exists in two isoforms. One is located in the mitochondrial matrix as in other eukaryotic cells, and the other is located in the chloroplast stroma (Randall et al., 1989).

The mtPDC provides the citric acid cycle with 2-carbon skeletons. mtPDC is regulated both by product inhibition (NADH and acetyl-CoA) and by protein phosphorylation/dephosphorylation (Randall et al., 1977; Rubin and Randall, 1977; Miernyk and Randall, 1987). The phosphorylation state of mtPDC is determined by the combined action of PDC kinase and phosphatase. It has been proposed that the mtPDC in vivo is less active in light compared to darkness (Budde and Randall, 1990; Gemel and Randall, 1992). This inhibition could be due to ammonium produced in the mitochondrial matrix by the Gly decarboxylase complex active in photorespiratory metabolism. Inactivation of mtPDC has been shown to be depressed when photorespiration was blocked (Gemel and Randall, 1992), and ammonium ions have been shown to be potential activators of mtPDC kinase (Schuller and Randall, 1989). The stromal cpPDC produces acetyl-building blocks for fatty acid, isoprenoid, and amino acid biosynthesis within the chloroplast. cpPDC is also regulated by product inhibition but is not phosphorylated (Elias and Givan, 1979; Camp and Randall, 1985; Liedvogel and Bäuerle, 1986). The PDC isoforms have similar substrate dependencies but differ in pH and magnesium optima (Williams and Randall, 1979; Treede and Heise, 1986; Hoppe et al., 1993). The requirement of cpPDC for higher pH and Mg^{2+} concentrations is in accord with an indirect regulation by light/dark transitions.

Specific activities of the two PDCs vary significantly between studies and species. These variations are most probably due to differences in organelle and enzyme purifications and methods used for assaying PDC activity. However, it cannot be ruled out that some of the variations are caused by species-specific differences in the phosphorylation state of mtPDC after organelle separation (Randall and Miernyk, 1990) or to different amounts of PDC protein in the organelles.

Only a few estimates have been published on the PDC isozyme proportions of the total cellular PDC activity in the same species. In pea, approximately 80% of the cellular PDC activity was confined to the mitochondria (Williams and Randall, 1979; Budde and Randall, 1990). A lower mtPDC compared to cpPDC activity was found in barley (Hoppe et al., 1993; Krömer et al., 1994). In spinach, a 10-fold higher mtPDC activity was found compared to cpPDC (Murphy and Stumpf, 1981). Notably, this comparison was based on organellar protein, which overestimates mitochondrial activity due to the low protein content of the mitochondrial compartment compared to the rest of the cell.

The differences in subcellular distribution noted between species could be due to developmental changes of the PDC enzyme capacities. The barley cpPDC has been proposed to be more active in the immature part of the leaf close to the leaf base, with decreasing activities along the developmental gradient of the growing leaf (Heintze et al., 1990). Similarly,

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Abbreviations: cpPDC, chloroplastic PDC; CS, citrate synthase; mtPDC, mitochondrial PDC; NADP-GAPDH, NADP-glyceraldehyde-3-phosphate dehydrogenase; PDC, pyruvate dehydrogenase complex; TPP, thiamine pyrophosphate.

young expanding leaves of dicotyledonous plants possess higher cpPDC activities compared to mature leaves (Williams and Randall, 1979; Murphy and Stumpf, 1981; Miernyk et al., 1985). This increased activity is proposed to correlate with high demands of lipids for membrane synthesis in the expanding cells. However, the developmental pattern of cpPDC is still uncertain, since several studies base enzyme activities on parameters that change significantly during leaf expansion (Murphy and Stumpf, 1981; Heintze et al., 1990; Hoppe et al., 1993).

In this study we have compared four plant species in order to present and characterize differences in subcellular distribution of the two PDC isoforms. The experiments were performed using leaf protoplasts because mesophyll cells are enriched in comparison to other cell types in the protoplast preparation. Thus, effects on enzyme distribution due to differences in activities in distinct cell types are minimized (Jellings and Leech, 1982). Also, fractionation of protoplasts with subsequent isolation of subcellular compartments gives low losses of protein compared to the total protoplast suspension, which makes it easier to follow the distribution of specific organelles using marker enzymes.

MATERIALS AND METHODS

Plant Material

Leaves were cultivated in growth chambers at light intensities of 150 to 300 μmol m^{-2} s^{-1} with a photoperiod of 12 h light, 22°C/12 h dark, 17°C. Barley (*Hordeum vulgare* L. cv Gunilla, Svalöf, Sweden), wheat (*Triticum aestivum* L. cv Starke II, Weibull, Sweden), and pea (*Pisum sativum* L. cv Kelvedon Wonder, Hammenhög, Sweden) were grown in a mixture of commercial potting soil and fertilized turf for 7 to 8 d (wheat, barley) or 14 to 16 d (pea). Spinach (*Spinacia oleracea* L. cv Medania, Weibull, Sweden) was grown in an aerated liquid growth medium with NO_3^- as the nitrogen source for 4 to 6 weeks before harvest.

Protoplast Preparation and Fractionation

Leaf protoplasts were prepared as described in detail earlier (Gardeström and Wigge, 1988), with the following modifications for pea and spinach: instead of removing the epidermis prior to digestion, leaves were cut into <5 mm^2 pieces and the pieces were digested for a longer time, 2 to 2.5 h. This prolonged digestion time did not affect the photosynthetic capacity of the cells but gave a somewhat higher yield of protoplasts. Protoplasts were kept on ice after preparation for a maximum of 2 h. For the developmental studies, protoplasts were prepared from consecutive parts along the primary barley leaf, starting from 0 to 0.75 cm (denoted "leaf section 1") and 0.75 to 1.5 cm ("leaf section 2") from the basal meristem and from each of the following 1.5 cm of the leaf. The last section used was from 9 to 10.5 cm from the leaf base (denoted "leaf section 8"). Protoplasts were not prepared from leaf tips.

Protoplasts were diluted to concentrations of 20 to 190 μg Chl mL^{-1} in dilution medium (0.25 M Suc, 0.25 M sorbitol, 10 mM Hepes [pH 7.2], 10 mM KCl, 0.5 mM $MgCl_2$, 0.2% [w/v] PVP, 0.06% [w/v] BSA [fatty acid free]). Some pea preparations were diluted in pea dilution medium containing 0.25 M Suc, 0.25 M sorbitol, 0.2% (w/v) PVP, 0.06% (w/v) BSA (fatty acid free), 5 mM EGTA, 10 mM Hepes (pH 8.2) to minimize adhesion of mitochondria and chloroplasts after protoplast breakage. Protoplasts were broken by passing them twice through a 20-μm nylon net fastened to a 2-mL plastic syringe. A part of the broken cell suspension was kept as the "total fraction." A 1.5-mL aliquot of the remaining broken cell suspension was centrifuged (swing-out rotor) in an Eppendorf tube at 300*g* for 3 min to pellet chloroplasts. This pellet, designated the "Cp fraction," was suspended in dilution medium to 1.5 mL. The supernatant fraction was centrifuged for 3 min at 13,000*g* to pellet mitochondria. The supernatant was taken as the "cytosol fraction" and the pellet, designated the "Mt fraction," was resuspended in 1.5 mL of dilution medium. The fractions were freeze-thawed (−20°C) once before enzyme measurements.

Oxygen Evolution Measurements

Photosynthesis by intact protoplasts was measured using a Hansatech O_2-electrode (Norfolk, UK). Assays were performed in a total volume of 1 mL with a protoplast concentration corresponding to 20 μg of Chl in dilution medium. Saturating CO_2 conditions were provided by adding 10 mM $NaHCO_3$. Photosynthesis was measured at a PFD of 500 μmol m^{-2} s^{-1} and 25°C until a steady-state rate was reached.

Determinations of Chl, Protein, and Protoplast Number

Chl was determined by the method of Arnon (1949). Measurements of protein concentration were done using a method based on the Lowry procedure (Peterson, 1977) with BSA as the standard protein. Estimations of cell concentration in the protoplast preparations were done using a Bürker counting chamber, where cell number per volume is estimated by light microscopy.

Enzyme Assays

Marker enzyme activities were measured at 25°C in all fractions. The chloroplast stromal marker NADP-GAPDH was measured according to Winter et al. (1982) with the modification that 4 mM DTT (replacing GSH) was included in the assay medium to ensure full activation of the enzyme. Fumarase (Hatch, 1978) and, in some experiments, CS were measured as mitochondrial matrix markers. The assay for CS activity contained 100 mM triethanolamine-KOH (pH 8.5), 0.1% (v/v) Triton X-100, 0.3 mM NAD, 4 mM malate, and 25 units/mL malate dehydrogenase. Reactions were initiated with 0.2 mM acetyl-CoA. PEP carboxylase (Gardeström and Edwards, 1983) was measured as a marker for the cytosol. For all marker enzymes the A_{340} of NAD(P)H was monitored on a DU-8 spectrophotometer (Beckman Instruments, Fullerton, CA).

mtPDC and cpPDC were assayed in the Mt and Cp fractions, respectively. Capacities were measured as described by Randall and Miernyk (1990) using a Sigma ZFP-22 dual-wavelength photometer (Biochem, Munich, Germany) monitoring NADH production at 334 nm against 405 nm at 25°C.

The assay medium contained, in a total volume of 600 μL, 0.16 M Tes-NaOH (pH 7.6 [mtPDC] or 8.0 [cpPDC]), 2 mM NAD, 0.2 mM TPP, 0.05% (v/v) Triton X-100, and $MgCl_2$ (1 [mtPDC] or 5 mM [cpPDC]). The assays were initiated by addition of 1 mM pyruvate (final concentration). Prior to the start of the reaction the background was monitored for 5 to 10 min until this was close to zero. Measurements were done with 15 to 30 μg of protein and were linear for at least 10 min. At protein amounts lower than 10 μg the activity of barley mtPDC was not linear, suggesting a dissociation of the complex at high dilutions, similar to what has been observed for the Gly decarboxylase complex (Ericson et al., 1984).

Corrections of PDC Distribution

The recovery of marker enzymes in the various fractions was between 80 and 120%. The enrichment and contamination of organelles in each fraction were estimated by calculating the ratio between the measured activity of each marker enzyme and the recovered total activity (Table I). Comparisons of PDC activities measured in the Cp and Mt fractions of different organelles and protoplast preparations were corrected for the loss of NADP-GAPDH and fumarase or CS activities, respectively. cpPDC activities were also corrected for contamination by mtPDC. This is possible because mtPDC has a close to optimal activity at pH 8.0 and 5 mM $MgCl_2$. In barley, wheat, and spinach the correction for mtPDC contamination of cpPDC capacities was negligible due to the low mtPDC activity. The cpPDC distribution in pea was altered from a distribution of 22 to 13% after recalculation. Correction of mtPDC activity for contaminating cpPDC activity was not done because the latter activity is inhibited approximately 50% at pH 7.6 (results not shown) and the contamination of NADP-GAPDH was ≤10% in the Mt fractions (Table I). The total PDC capacity in the cell was calculated by adding the specific capacities of mtPDC and cpPDC. Data shown are from two or more protoplast preparations from each species or barley leaf section. SE values are included only if measurements are from more than two preparations.

RESULTS

Protoplast Preparation and Fractionation

Barley and wheat protoplasts showed photosynthetic activities of 100 to 150 μmol O_2 evolved mg^{-1} Chl h^{-1}. Pea and spinach preparations had lower yields of cells compared to the monocotyledonous species and showed lower photosynthetic activities, approximately 50 μmol O_2 mg^{-1} Chl h^{-1}. Protoplasts from barley, wheat, spinach, and some pea preparations were fractionated in dilution medium (pH 7.2) containing 0.5 mM $MgCl_2$. The recovery of the chloroplast marker enzyme in the Cp fraction was about 70 to 80% (Table I). Contamination of mitochondria in this fraction was about 10% for barley and 20 to 30% for wheat and spinach. In the Mt fraction the recovery of the mitochondrial marker enzyme was 40 to 60% and the contamination by the chloroplastic marker was ≤10%. For all species tested, 80% of the cytosolic marker PEP carboxylase was recovered and 10 to 20% of both the NADP-GAPDH and fumarase or CS activities were found in the cytosolic fraction (results not shown). Fractionation of pea protoplasts could not be accomplished satisfactorily in the same medium as barley protoplasts, since mitochondrial contamination of the Cp fraction was high (41%). By increasing the pH to 8.2 and removing endogenous Mg^{2+} by adding 5 mM EGTA to the dilution medium, this was reduced, giving an 87% recovery of chloroplasts and a 20% contamination by mitochondria (Table I). However, the pea dilution medium destabilized the remaining mitochondria, decreasing the yield (18 ± 7.3%) of matrix proteins significantly in the Mt fraction. Thus, pea cpPDC was measured in a Cp fraction derived from fractionation in pea breaking medium, whereas mtPDC was measured in a Mt fraction obtained after protoplast fractionation in dilution medium (pH 7.2, 0.5 mM $MgCl_2$). The change of medium did not

Table I. *Marker enzyme distributions and specific PDC activities in Mt and Cp fractions of different species*

Marker enzymes are presented as percentage of the total recovered activity. A total activity of 100% corresponds to: NADP-GAPDH, 388 ± 107; CS, 5.6 ± 0.9; and fumarase, 14.3 ± 2.8 μmol mg^{-1} Chl h^{-1}. Pea protoplasts were fractionated in two different media (see "Materials and Methods"); the Mt fraction is from protoplast breakage in dilution medium, and the Cp fraction is from fractionation in pea dilution medium. PDC activities are corrected as described in "Materials and Methods."

Species	Fraction	No. of Fractionations (No. of Protoplast Preparations)	NADP-GAPDH	CS or Fumarase	mtPDC	cpPDC
			% of recovered		μmol mg^{-1} Chl h^{-1} (% distribution)	
Barley	Mt	9 (6)	9.8 ± 3.6	61 ± 19	0.21 ± 0.14 (15)	
	Cp	8 (7)	67 ± 13	9.8 ± 4		1.2 ± 0.14 (85)
Pea	Mt	7 (5)	6.4 ± 4.4	38 ± 16	1.36 ± 0.47 (87)	
	Cp	5 (3)	87 ± 1.5	20 ± 4.6		0.21 ± 0.07 (13)
Wheat	Mt	4 (2)	3 ± 1.2	59 ± 11.6	0.08 ± 0.016 (13)	
	Cp	5 (2)	77 ± 16	21 ± 9.3		0.52 ± 0.15 (87)
Spinach	Mt	2 (2)	4.5	54	0.17 (22)	
	Cp	2 (2)	73	34		0.60 (78)

affect the total corrected PDC capacity. Contamination of spinach chloroplasts by mitochondria could not be improved by fractionation in the pea dilution medium (results not shown).

PDC Distribution in Different Species

PDC isoforms were measured under conditions optimal for the specific enzyme in the enriched organelle fractions. Assay conditions were not changed for the species studied, since published pH and magnesium optima for different species have not shown any significant differences (Williams and Randall, 1979; Treede and Heise, 1986; Hoppe et al., 1993). All of the measured PDC capacities were fully dependent on pyruvate, NAD, and CoA. Removal of TPP from the assay medium decreased the mitochondrial enzyme activity more than that of the chloroplastic isoform (results not shown). In barley, the apparent K_m values for pyruvate, NAD, CoA, and TPP were similar to earlier published data on pea (Camp et al., 1988; Randall et al., 1989) (results not shown).

The corrected specific mtPDC and cpPDC activities based on total cellular Chl are shown in Table I. There were significant differences in the capacities of the PDC isoforms in the species tested. Pea mtPDC had an activity about 6 times higher than that in barley and spinach and 17 times higher than that in wheat. cpPDC capacities also varied in the different species, but these variations were smaller than those found for mtPDC. Barley cpPDC had about twice the capacity compared to that in wheat and spinach, and 6 times higher compared to that in pea. Total PDC capacity in the protoplasts could not be obtained due to high background activities. The use of an alternative method to measure PDC activity could avoid this problem (Budde and Randall, 1990). However, optimal assay conditions for both isoforms would not be possible to obtain. Instead, total PDC activities were estimated by simply adding the corrected mtPDC and cpPDC rates. Pea and barley had similar total PDC activities, 1.6 and 1.4 μmol NADH mg^{-1} Chl h^{-1}, respectively, whereas in wheat and spinach the activities were lower, 0.6 and 0.8 μmol NADH mg^{-1} Chl h^{-1}, respectively. The distribution in barley, wheat, and spinach was similar, with a higher cpPDC (78–87%) than mtPDC (13–22%) capacity. Pea had the opposite distribution, with 13% cpPDC and 87% mtPDC (Table I).

PDC Isoforms in the Developing Barley Leaf

The influence of cell age on the specific activities and subcellular distribution of the PDC isoforms was studied in protoplasts from different parts of 7-d-old barley leaves. The protoplasts were fractionated and marker enzymes, as well as PDC capacities, were measured (Fig. 1). Capacities were related to cell number instead of Chl, since the latter increases during cell maturation. This increase was correlated with the rise in photosynthetic capacity (Fig. 1A). The development of NADP-GAPDH activity followed the photosynthetic activity of the cells. This Calvin cycle enzyme had at least a 100 times higher activity than cpPDC (Fig. 1B). Chl content, photosynthesis, and NADP-GAPDH activity all decreased in the oldest section of the leaves close to the tip. The mitochondrial enzymes fumarase and CS showed relatively constant activities with increasing cell age (Fig. 1C). The capacities of these two citric acid cycle enzymes were in the same range, but 10 to 20 times higher than the mtPDC activity. cpPDC has a significantly higher specific activity than mtPDC, 100 to 150 compared to 20 to 30 pmol NADH $cell^{-1}$ h^{-1}. Both the cpPDC and mtPDC showed the largest deviation in activities in the youngest cells. This might be due to difficulties in preparing

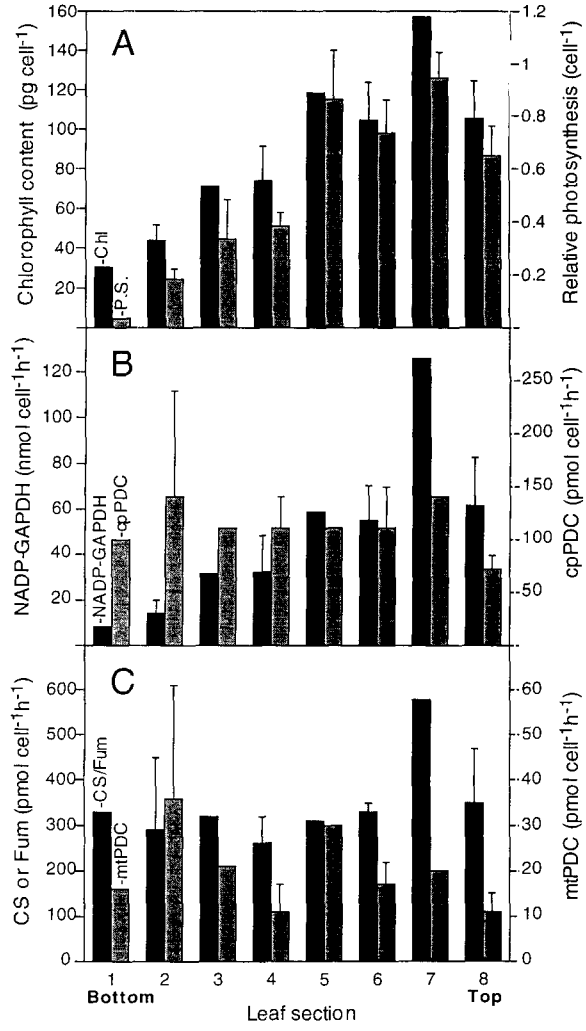

Figure 1. Developmental patterns of cellular activities in a barley leaf. Starting from the bottom of the leaf, section 1 corresponds to 0 to 0.75 cm from the leaf base, section 2 from 0.75 to 1.5 cm, and sections 3 to 8 each constitute a subsequent 1.5-cm leaf section. Panel A shows the Chl amount (pg) per cell (■). Sections 1, 3, 5, and 7 are from two experiments, whereas the remaining sections are from four experiments. Relative photosynthesis per cell (P.S.) (▨) is from three experiments. A relative rate of 1 corresponds to 15 to 20 pmol O_2 evolved $cell^{-1}$ h^{-1}. Development of the chloroplastic enzymes NADP-GAPDH (■) and cpPDC (▨) is shown in panel B and the mitochondrial enzymes CS or fumarase (Fum) (■) and mtPDC (▨) in panel C. Activities of CS and fumarase were in the same range; thus, the values shown are the means of the rates of both enzymes. Results for sections 1, 3, 5, and 7 are the means of two experiments and sections 2, 4, 6, and 8 are means ± SE of four experiments.

cells of the same age in the most immature part of the leaf, since the developmental gradient is steep in these sections. This gradient is influenced by differences in growth conditions between different experiments and between individual leaves. In the sections close to and above the middle of the leaves, cells in larger parts of the leaf are similar in developmental stage. Since both PDC isoforms had relatively constant capacities in all leaf sections (Fig. 1, B and C), the distribution of PDC activity between the two isoforms does not change significantly during barley leaf growth.

DISCUSSION

In this study we demonstrate that the subcellular distribution of mtPDC and cpPDC activities differs between pea on the one hand, and barley, wheat, and spinach on the other. These differences in distribution were not due to species-specific variations in substrate dependencies, pH, or magnesium optima of the two isoforms. Neither were differences in PDC distributions between pea and barley due to diverging developmental patterns of the PDC isoforms in barley leaves. This could have been expected because earlier reports of cpPDC development in barley (Heintze et al., 1990) have shown declining cpPDC capacities with leaf age. mtPDC capacity, on the other hand, together with the citric acid cycle enzymes CS and fumarase (Fig. 1C) and the respiratory chain enzyme Cyt *c* oxidase and the matrix protein glutamate dehydrogenase (Tobin et al., 1988), were more constant during leaf growth. This might reflect the need for mitochondrial respiration during leaf development. However, we found that cpPDC capacity per cell was relatively constant in the developing barley leaf (Fig. 1B) and, thus, not correlated with possibly higher rates of fatty acid synthesis in the young cells. Neither did the cpPDC capacity correlate with chloroplast development when Chl synthesis and photosynthesis increased (Fig. 1A), and several stromal enzymes, including NADP-GAPDH (Fig. 1B; Sibley and Anderson, 1989), phosphoglycerate kinase (Shah and Bradbeer, 1991), and Fru-1,6-bisphosphatase (Sibley and Anderson, 1989), had higher capacities toward the leaf tip compared to the leaf base. The higher activity of NADP-GAPDH compared to cpPDC is in line with the function of the former enzyme in primary carbon fixation and the function of the latter in biosynthetic processes.

Questions that remain to be answered are whether the species differences in subcellular distribution of the two PDC isozymes are due to variations between species in the phosphorylation state of mtPDC (Randall and Miernyk, 1990) or to actual differences in PDC protein amounts. Pea mtPDC could, after organelle isolation, be less phosphorylated than the barley, wheat, and spinach enzymes. However, care was taken to ensure that growth of leaves and protoplast preparations and storage of protoplasts and organelles were done similarly for all species. Also, deactivation or activation of mtPDC after organelle preparation could probably occur only to a limited extent, since substrate ATP and the kinase activator NH_4^+ (Schuller and Randall, 1989), as well as substrates that inhibit mtPDC kinase, pyruvate, NADH, and acetyl-CoA (Randall et al., 1989), are low in the Mt fraction due to the high dilution of the mitochondria. Also, it has been shown that barley mtPDC, obtained after a rapid filtration of protoplasts, can be deactivated by incubation for 10 min with 1 mM ATP to approximately 40 to 50% of the original activity (Krömer et al., 1994). However, if differences in phosphorylation status are found between species, it is still questionable whether the subcellular distribution will change significantly. For example, barley mtPDC would have to be activated to nearly 500% in order to obtain a subcellular PDC distribution similar to pea. Krömer et al. (1994) could increase mtPDC activity by 50% when isolated barley mitochondria were incubated for 10 min with 20 mM $MnCl_2$. In the same study, $MgCl_2$ incubation did not stimulate mtPDC activity as has been found for pea. This might indicate possible differences in regulation of the mtPDC phosphatases from pea and barley.

Activities of mtPDC based on organellar protein vary among species, indicating that species variations in PDC distribution could be due to differences in mtPDC protein amounts in the mitochondria. In this study the rate of mtPDC activity in isolated barley mitochondria amounted to 4.6 μmol mg^{-1} protein h^{-1}, which is half of the activity measured in pea (Budde and Randall, 1990) and three times that in spinach (Rao and Randall, 1980). Interestingly, this trend is repeated by the mtPDC capacities, based on total cellular Chl, assayed in fractionated protoplasts. Rates of cpPDC activity compared among different species were more similar than found for mtPDC (Table I). However, quantification of PDC protein amounts in the organelles must be done to determine if the apparent species differences in mtPDC capacities are due to differences in the phosphorylation state of mtPDC and/or protein amounts.

Since questions still remain concerning any metabolic purpose of the apparent distribution differences among species, studies on regulatory mechanisms of the PDC isoforms are of interest. Our data suggest that there are differences in acetyl-CoA production among species and intracellular compartments. In the literature there are some uncertainties whether or not the chloroplast in all species is fully self-providing with respect to acetyl-CoA by its PDC isoform or if acetyl-CoA is supplied from other compartments of the cell (Givan, 1983). The substrate pyruvate for cpPDC could be provided by a stromal glycolytic pathway starting with 3-phosphoglycerate (Murphy and Leech, 1978; Hoppe et al., 1993). Alternatively, malate could be imported from the cytosol and converted into pyruvate by a stromal NADP-malic enzyme (Smith et al., 1992), or pyruvate could be imported directly to the stroma (Smith et al., 1992). Furthermore, acetyl-CoA could be produced in the stroma from acetate by an acetyl-CoA synthetase (Kuhn et al., 1981). This acetate could be synthesized in the mitochondria by the combined action of mtPDC and an acetyl-CoA hydrolase (Murphy and Stumpf, 1981; Zeiher and Randall, 1990). Also, acetyl-CoA could be shuttled directly between the mitochondria and chloroplasts by a carnitine/acetyl carnitine exchange system across the mitochondrial (Burgess and Thomas, 1986) and chloroplast (McLaren et al., 1985) inner membranes. The low mtPDC to cpPDC capacity found in barley leaves raises the question of whether enough acetyl-CoA is produced by mtPDC to provide substrate for both CS and a possible acetyl-CoA hydrolase. In the light when fatty acid synthesis is

occurring (Browse et al., 1981), the mtPDC of pea has been shown to be inactivated (Budde and Randall, 1990; Gemel and Randall, 1992). This might indicate that the illuminated chloroplast must be at least partially self-providing with respect to substrate for cpPDC, whereas in darkness acetyl-CoA used in the stroma is produced to a greater extent in the mitochondria.

In conclusion, the species-specific differences in subcellular PDC distribution could be related to differences in regulation of the PDC isoenzymes in chloroplasts and mitochondria. Further investigations are needed to clarify this possibility.

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