Comparative Studies of Enzymes Related to Serine Metabolism in Higher Plants[1]

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Abstract. The following enzymes related to serine metabolism in higher plants have been investigated: 1) D-3-phosphoglycerate dehydrogenase, 2) phosphohydroxypyruvate:L-glutamate transaminase, 3) D-glycerate dehydrogenase, and 4) hydroxypyruvate:L-alanine transaminase. Comparative studies on the distribution of the 2 dehydrogenases in seeds and leaves from various plants revealed that D-3-phosphoglycerate dehydrogenase is widely distributed in seeds in contrast to D-glycerate dehydrogenase, which is either absent or present at low levels, and that the reverse pattern is observed in green leaves.

The levels of activity of the 4 enzymes listed above were followed in different tissues of the developing pea (*Pisum sativum*, var. Alaska). In the leaf, from the tenth to seventeenth day of germination, the specific activity of D-glycerate dehydrogenase increased markedly and was much higher than D-3-phosphoglycerate dehydrogenase which remained relatively constant during this time period. Etiolation resulted in a decrease in D-glycerate dehydrogenase and an increase in D-3-phosphoglycerate dehydrogenase activities. In apical meristem, on the other hand, the level of D-3-phosphoglycerate dehydrogenase exceeded that of D-glycerate dehydrogenase at all time periods studied. Low and decreasing levels of both dehydrogenases were found in epicotyl and cotyledon. The specific activities of the 2 transaminases remained relatively constant during development in both leaf and apical meristem. In general, however, the levels of phosphohydroxypyruvate:L-glutamate transaminase were comparable to those of D-3-phosphoglycerate dehydrogenase in a given tissue as were those for hydroxypyruvate:L-alanine transaminase and D-glycerate dehydrogenase.

Two separate pathways for serine biosynthesis in animal systems have been established (9, 18, 29, 31). The individual reactions in the pathway utilizing phosphorylated intermediates (phosphorylated pathway) are given by reactions 1 to 3 and those involving nonphosphorylated compounds (nonphosphorylated pathway) are shown in reactions 4 and 5: 1) D-3-P-glycerate + $DPN^+ \longleftrightarrow$ P-hydroxypyruvate + DPNH + H^+, 2) P-hydroxypyruvate + L-glutamate $\longleftrightarrow$ L-P-serine + α-ketoglutarate, 3) L-P-serine + $H_2O \rightarrow$ L-serine + P_i, 4) D-glycerate + $DPN^+ \longleftrightarrow$ hydroxypyruvate + DPNH + H^+, 5) hydroxypyruvate + L-alanine $\longleftrightarrow$ L-serine + pyruvate. Evidence for the occurrence of both of the above pathways for serine metabolism in plant systems has been reported and is discussed below. Relatively few of these studies, however, have been carried out on the individual enzymatic reactions in either of the 2 routes.

The phosphorylated pathway, described above in reactions 1 to 3, was first demonstrated in higher plants by Hanford and Davies (5) who showed the over-all conversion of D-3-P-glycerate to L-P-serine and L-serine in homogenates of pea epicotyls. In addition, the results of *in vivo* $^{14}CO_2$ labeling experiments during short term photosynthesis are consistent with a functional phosphorylated pathway for serine formation in many plant preparations including spinach chloroplasts (3), tobacco leaves (6), and algae (7). Furthermore, the occurrence in wheat germ of all of the enzymes of the phosphorylated pathway, *i.e.* PGDH[4], P-hydroxypyruvate:L-glutamate transaminase, and phosphoserine phosphatase, has been demonstrated in this laboratory (unpublished observations).

The second route for serine formation in plants is *via* the glycolate pathway, also known as the glyoxylate-serine pathway (16, 28). This pathway, by which glycolate is converted to sugars in photosynthesizing tissues, has been proposed on the basis of *in vivo* studies with ^{14}C-labeled substrates and

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[4] Abbreviations used are: PGDH = D-3-phosphoglycerate dehydrogenase; GDH = D-glycerate dehydrogenase.

involves the following sequence of reactions: glycolate ⇌ glyoxylate ⟷ glycine ⟷ serine ⟷ D-glycerate → D-3-P-glycerate → → → → hexoses. Much of the literature on the metabolism of intermediates of this pathway in plants has been reviewed in recent papers from the laboratories of Cossins (20) and Tolbert (23).

Early studies on glycolate and glyoxylate metabolism in leaf tissues were reported in 1962 (16, 28). Since that time, numerous detailed studies utilizing different ^{14}C-labeled intermediates of the glycolate pathway have been carried out with a wide variety of plant tissues including wheat leaves (25, 26, 27), pea leaves (15), tobacco leaves (6) pea roots, pea cotyledons, corn roots, corn coleoptiles, and sunflower cotyledons (20). In all of these investigations serine has been implicated as a key intermediate in the over-all path of carbon from glycolate to carbohydrate. Available evidence from the above *in vivo* studies suggests that the pathway from serine to sucrose is *via* hydroxypyruvate, D-glycerate and D-3-P-glycerate.

Although the nonphosphorylated pathway (described above in reactions 4 and 5) has not been implicated as a functional route for serine formation from carbohydrate in plants, both enzymes involved, *i.e.* GDH (21) and hydroxypyruvate:L-alanine transaminase (30) have been demonstrated in a variety of plant tissues. Both enzymes show essentially the same distribution of activity in that they are widely distributed in green leaves with little or no activity in seeds or tubers. Developmental studies carried out with peas and wheat have confirmed the fact that GDH is primarily a leaf enzyme and that the main increase in enzymatic activity during growth is associated with maximal leaf development (22).

In view of the alternate routes for serine metabolism in plants, a comparative study of the enzymes of the phosphorylated and nonphosphorylated pathways was carried out to investigate the relative significance of the 2 routes. The distribution of PGDH in seeds and leaves from various plant sources has been compared to that of GDH. In addition, the relative levels of activity of the dehydrogenases and transaminases in different parts of the developing pea plant have been determined and are reported.

Materials and Methods

Reagents and Substrates. Crystalline lactate dehydrogenase, glutamate dehydrogenase, pyruvate kinase, DPN, DPNH, ATP, D-3-P-glycerate, pyruvate, pyridoxal phosphate, tris-HCl, and ignited sea sand were obtained from Sigma Chemical Company. Crystalline phosphoglycerate mutase and enolase were purchased from C. F. Boehringer and Soehne, GmbH, Mannheim. GDH was purified from spinach leaves to step 4 as outlined elsewhere (19); aliquots of the ammonium sulfate residues, which were stored at −20°, were dissolved in 0.05 M phosphate buffer, pH 7.4, as needed and freed of ammonium ions by dialysis against the same buffer before use. D-Glycerate and hydroxypyruvate were prepared and assayed as described previously (29). The cyclohexylammonium salt of the dimethyl ketal of P-hydroxypyruvate (Calbiochem.) was converted to the free acid using the method of Ballou (1). Other reagents were commercial preparations of the highest purity available. Stock solutions of all reagents were prepared at neutrality as their potassium salts unless otherwise indicated.

Plant Materials. In the distribution studies, plants were either grown in the laboratory or obtained from commercial sources. All seeds were purchased from local dealers. For the developmental studies, pea seeds, *Pisum sativum* (var., Alaska), were soaked overnight in tap water and then planted about 3 cm deep in vermiculite for growth. Temperature was kept constant at 30° and a daily photoperiod of 12 hr was maintained except for dark-grown plants. Water was supplied daily to all plants. At the indicated times after planting, the peas were harvested and their mature leaves, apical meristems, cotyledons, and epicotyls (the latter being defined as that area of the shoot extending from its attachment to the cotyledons to the level directly below the first lateral shoot) were dissected out and immediately frozen in a container immersed in an acetone-dry ice bath.

Enzyme Preparations. All operations were carried out at 4° unless otherwise stated.

In the distribution studies, plant materials were prepared as described by Stafford *et al.*, (21) except that the extractions were made with 0.1 M phosphate buffer, pH 7.4, containing 0.003 M EDTA and 0.0001 M 2-mercaptoethanol.

The following conditions were used in the developmental studies. Frozen tissues were ground with sea sand in a mortar and pestle with 1:3 (w/v) ratio of 0.01 M tris-HCl buffer, pH 7.4, containing 0.001 M EDTA and 0.0001 M 2-mercaptoethanol. The slurry was filtered through cheese cloth prior to centrifugation at 35,000*g* for 30 min. The resultant supernatant solution was centrifuged at 105,000*g* for 30 min and the final supernatant brought to 80% saturation by the slow addition, with stirring, of the calculated amount of solid ammonium sulfate. The precipitate was collected by centrifugation and redissolved in 0.01 M phosphate buffer, pH 7.4, containing 0.001 M EDTA and 0.0001 M 2-mercaptoethanol, in a 1:1 (v/w) ratio of the original tissue weight. This preparation was used for the assays of the dehydrogenases. Before use in the assays for the transaminases and for D-glycerate kinase, the enzyme preparation was dialyzed against the same phosphate buffer with regular changes until the dialysate gave a negative reaction to Nessler's reagent.

In control experiments, the supernatant solutions from the 80% ammonium sulfate fraction were

assayed for dehydrogenase and transaminase activities. None were detected. All enzyme assays were performed on the same day that the plants were harvested. Protein concentrations were determined by the method of Lowry *et al.*, (13) using bovine serum albumin as the standard.

Standard Assays for GDH and PGDH. The standard assay conditions described previously were used for GDH (29) and PGDH (31). Initial velocities were measured using a Gilford 2000 recording spectrophotometer. Assays were carried out in triplicate on each sample and the average value reported.

Standard Assay for P-Hydroxypyruvate:L-Glutamate Transaminase. Detailed conditions for the determination of this transaminase have been published previously (24). The present studies were carried out essentially in the same manner except that the complete reaction mixture contained: sodium borate buffer, pH 8.2, 100 μmoles; pyridoxal phosphate, 2.5×10^{-2} μmoles; EDTA, 50 μmoles; L-glutamate, 10 μmoles; 2-mercaptoethanol, 0.05 μmoles; and P-hydroxypyruvate, 5 μmoles, in a total volume of 1.4 ml. The same controls as used previously, *i.e.* zero time control, internal α-ketoglutarate standard, and complete reaction mixtures in which either P-hydroxypyruvate or L-glutamate was omitted (*cf.* 24), were carried out under the above conditions with water added where substrates were omitted. The reaction was stopped by the addition of 0.1 ml of 4.5 N perchloric acid and precipitated protein was removed by centrifugation. Aliquots of the deproteinized solutions (1.0 ml) were diluted with 0.25 ml of water before the addition, with vigorous stirring, of 0.25 ml of 1 N KOH. The solutions were chilled and insoluble potassium perchlorate was removed by centrifugation. Aliquots of the resulting supernatant solutions were assayed for α-ketoglutarate. The determination of this α-keto acid and all other conditions were identical to those outlined earlier (24).

Standard Assay for Hydroxypyruvate:L-Alanine Transaminase. The method used for the determination of this transaminase is described in detail below. In general, the procedure involves the measurement of the product of the reaction, pyruvate, in the presence of unreacted hydroxypyruvate. Both of these α-keto acids are substrates for lactate dehydrogenase (14), but only the latter compound is a substrate for spinach GDH (8). In the assay procedure, unreacted hydroxypyruvate is quantitatively reduced in the presence of excess DPNH and spinach GDH prior to the spectrophotometric determination of pyruvate with lactate dehydrogenase.

In the standard assay for the transaminase, varying levels of enzyme were preincubated for 10 min at 37° with sodium borate buffer, pH 8.2, 100 μmoles; pyridoxal phosphate, 2×10^{-2} μmoles; and L-alanine, 10 μmoles. The reaction was started by the addition of 6 μmoles of hydroxypyruvate to give a final volume of 1.4 ml. The reaction was terminated after a 60 min incubation at 37° by the addition of 0.1 ml of 4.5 N perchloric acid.

The enzymatic activities of all tissues were routinely determined at 4 levels of enzyme and the linear relationship between enzyme concentration and pyruvate produced was used in the calculation of specific activity. A complete reaction mixture that was stopped at zero time was used to monitor for the presence of pyruvate in the enzyme solution. Additional controls included complete reaction mixtures except for the following: A) enzyme, to determine possible nonenzymatic transamination as well as nonenzymatic loss of hydroxypyruvate; B) alanine, to measure enzymatic loss of hydroxypyruvate; and C) hydroxypyruvate, to determine production of pyruvate from alanine by other possible reactions. Finally, a complete reaction mixture, in which both substrates were omitted and 1.0 μmole of pyruvate was added, was used as an internal standard to monitor for pyruvate recovery.

Following deproteinization, 0.25 ml of 1 N KOH was added, with vigorous stirring, to 1.0 ml aliquots of the supernatant solutions. After chilling, insoluble potassium perchlorate was removed by centrifugation. Aliquots of the resulting supernatant solutions were diluted 1:1 (v/v) with 1.0 M phosphate buffer, pH 7.4, prior to the spectrophotometric determination of unreacted hydroxypyruvate and of the pyruvate produced by transamination.

Aliquots (0.5 ml) of the above diluted solutions were transferred to cuvettes which contained 100 μmoles of phosphate buffer, pH 7.4, and 1.22 μmoles of DPNH in a final volume of 2.8 ml. The absorbancy at 340 mμ was measured with a Gilford 2000 spectrophotometer using a water blank before and after the addition of 0.1 ml of spinach GDH (40×10^3 units). Due to the favorable equilibrium of this reaction, $K_{eq} = 0.33 \times 10^{12}$ (8), any hydroxypyruvate present is reduced immediately with a concomitant decrease in DPNH concentration. A control assay mixture containing only buffer, water, DPNH, and spinach GDH was used to monitor for any nonspecific oxidation of DPNH. After corrections for volume changes and any changes in absorbancy of control assay mixtures, unreacted hydroxypyruvate concentration is calculated from the observed change in absorbancy at 340 mμ using the molar extinction coefficient of DPNH.

Following the above step, 0.1 ml of a solution of crystalline lactate dehydrogenase (0.25 mg) was added to the contents of the control and experimental cuvettes. Since excess DPNH is still present, any pyruvate in the reaction mixtures is reduced to lactate with the simultaneous oxidation of DPNH. The changes in absorbancy at 340 mμ with control and experimental reaction mixtures are used for the calculation of pyruvate concentration as outlined above for hydroxypyruvate.

Certain points about the assay procedure should be noted. The sum of the pyruvate produced and the unreacted hydroxypyruvate recovered should be

equal to the 6 μmoles of hydroxypyruvate added at the start of the reaction. In most experiments, this recovery was 90 %. The controls carried out in the transaminase assay that permitted a measure of hydroxypyruvate recovery, *i.e.* nonenzyme blank, zero time control, and complete reaction mixture minus alanine, demonstrated that under the conditions of the assay there was routinely a small nonenzymatic loss of hydroxypyruvate. In the control in which substrates were omitted and pyruvate added in the transaminase assay, recoveries of pyruvate by the spectrophotometric assay were greater than 90 %.

Assay for D-*Glycerate Kinase.* The spectrophotometric procedure described by Lamprecht *et al.*, (11) was used for the assay of this enzyme.

Specific Activities of Enzyme. One unit of enzyme activity for both dehydrogenases and transaminases is defined as that amount of enzyme that catalyzes the formation of 1 mμmole of product per min under the standard conditions of assay. Specific activity is units per mg of protein.

Results

Distribution Studies. The levels of activity of PGDH and GDH found in a variety of seeds and leaves are summarized in table I. PGDH is widely distributed in seeds in contrast to GDH which is either absent or present at very low levels. In those seeds in which both activities were detected, the level of PGDH greatly exceeds that of GDH. In contrast to seeds, mature leaves show the opposite distribution of the 2 dehydrogenases. Since PGDH is primarily associated with seeds and GDH with leaves, the activities of the 2 dehydrogenases in different tissues were compared in germinating peas.

Developmental Studies on GDH and PGDH. The levels of activity of both dehydrogenases in leaves during development of peas are given in table II. When plants are grown in the light, PGDH activity remains relatively constant within the time period studied. On the other hand, the specific activity of GDH shows a marked increase between 10 and 12 days, after which time there is a gradual increase to a relatively constant level. These results confirm observations made by Stafford and Magaldi (22) who reported similar changes in GDH activity at this stage of development. The over-all trend of increasing activities of GDH, as compared to PGDH, during leaf development is seen more clearly when the ratios of the respective activities are used as the criterion of change. In contrast to the results obtained in the light, a reciprocal trend is observed in etiolated pea leaves as reflected by an increase in the PGDH:GDH ratios.

The levels of activity of the 2 dehydrogenases in apical meristem during development (table III) differ significantly from those in leaf. PGDH activity is several fold higher and GDH is significantly lower in apical meristem than in leaf. However, as in leaf, GDH shows an increase between 10 and 12

Table II. *Specific Activities of 3-Phosphoglycerate and* D-*Glycerate Dehydrogenases in Pea Leaves During Development*

Experimental conditions as described in table I.

Age and growth conditions	GDH	PGDH	PGDH : GDH
	mμmoles per mg protein per min		*ratio*
10 days, light	10.4 ± 1.6[1]	3.5 ± 0.9	0.34
11 " "	23.0 ± 1.8	3.3 ± 0.3	0.14
12 " "	33.7 ± 3.2	3.1 ± 0.5	0.09
14 " "	31.4 ± 1.8	2.2 ± 0.5	0.07
15 " "	36.5 ± 1.1	2.2 ± 0.4	0.06
17 " "	38.9 ± 1.6	2.1 ± 0.3	0.05
12 days, dark	7.7 ± 0	6.9 ± 0	0.90
16 " "	7.4 ± 0.3	5.8 ± 0	0.78

[1] Mean deviation of results from at least 3 separate growth experiments.

Table I. *Distribution of 3-Phosphoglycerate and* D-*Glycerate Dehydrogenases in Plant Seeds and Leaves*

The procedures used for the enzyme preparation, standard conditions for assay and other experimental details are described under Materials and Methods.

Source	Seed		Leaf	
	Specific activities of:			
	PGDH	GDH	PGDH	GDH
	mμmoles per mg protein per min			
Pea (*Pisum sativum*)	10.4	<0.3	2.1	40.0
Soybean (*Glycine max*)	3.4	0.4	5.8	24.2
Snap Bean (*Phaseolus vulgarus*)	17.0	1.2	2.6	9.4
Mung Bean (*Phaseolus aureus*)	4.5	0	<0.3	8.9
Oat (*Avena sativa*)	2.5	0	<0.3	18.1
Wheat (*Triticum aestivum*)	<0.3	<0.3	...	...
Wheat Germ	7.6	<0.3	...	...
Beet (*Beta vulgaris*)	...	...	<0.3	23.5
Parsley (*Petroselinum crispum*)	...	...	<0.3	14.4
Spinach (*Spinacia oleracea*)	...	...	1.5	27.8
Radish (*Raphanus sativas*)	...	...	<0.3	12.7
Endive (*Cichorium endiva*)	...	...	<0.3	5.4

Table III. *Specific Activities of 3-Phosphoglycerate and D-Glycerate Dehydrogenases in Apical Meristem During Pea Development*

Experimental conditions as described in table I.

Age and growth conditions	GDH	PGDH	PGDH : GDH
	mμmoles per mg protein per min		*ratio*
10 days, light	2.8 ± 0.2[1]	12.9 ± 0	4.6
11 " "	8.1 ± 1.7	14.4 ± 1.6	1.8
13 " "	8.3 ± 1.1	15.4 ± 1.3	1.9
14 " "	6.4 ± 1.0	16.0 ± 0.9	2.5
15 " "	5.5 ± 0.9	15.4 ± 0.6	2.8
16 " "	4.5 ± 0.5	14.5 ± 0.3	3.2
17 " "	4.3 ± 0	16.0 ± 0.1	3.7
12 days, dark	2.7 ± 0.1	14.6 ± 0.4	5.4
14 " "	2.4 ± 0.3	10.4 ± 0.4	4.3
16 " "	3.2 ± 0.1	11.4 ± 0.5	3.6

[1] Mean deviation of results from at least 3 separate growth experiments.

Table IV. *Specific Activities of 3-Phosphoglycerate and D-Glycerate Dehydrogenases in Pea Epicotyls During Development*

Experimental conditions as described in table 1.

Age and growth conditions	GDH	PGDH	PGDH : GDH
	mμmoles per mg protein per min		*ratio*
10 days, light	0.7 ± 0.1[1]	1.3 ± 0.5	1.9
11 " "	1.0 ± 0.1	3.3 ± 0.6	3.3
13 " "	0.8 ± 0.2	2.8 ± 0.4	3.5
15 " "	0.8 ± 0.1	1.9 ± 0.2	2.4
16 " "	0.7 ± 0.1	1.2 ± 0.3	1.7

[1] Mean deviation of results from at least 3 separate growth experiments.

Table V. *Specific Activities of 3-Phosphoglycerate and D-Glycerate Dehydrogenases in Pea Cotyledons During Development*

Experimental conditions as described in table I.

Age and growth conditions	GDH	PGDH	PGDH : GDH
	mμmoles per mg protein per min		*ratio*
10 days, light	1.4 ± 0.3[1]	4.7 ± 0.7	3.4
11 " "	1.6 ± 0.2	2.9 ± 0.5	1.8
12 " "	1.7 ± 0.3	1.8 ± 0.4	1.1
13 " "	1.3 ± 0.3	1.2 ± 0.4	0.9
14 " "	1.5 ± 0.3	0.5 ± 0.1	0.3
15 " "	1.5 ± 0.3	...	...
16 " "	1.5 ± 0.3	...	...
17 " "	1.5 ± 0.3	...	...

[1] Mean deviation of results from at least 3 separate growth experiments.

days. Etiolation appears to have little or no effect on PGDH whereas GDH activity decreases to values lower than those corresponding to the same time period when plants are grown in the light.

In epicotyls during development (table IV), GDH remains constant throughout the time period studied. However, PGDH shows a decrease in activity after the eleventh day, probably reflecting the cessation of growth in this region of the plant as development proceeds. The last tissue studied with respect to the activities of the 2 dehydrogenases during growth was the cotyledon (table V). The levels of GDH activity remain essentially constant until the seventeenth day of growth after which time activity of this enzyme could not be detected. The level of PGDH activity decreased with time until undetectable after 15 days of growth.

The levels of both dehydrogenases observed in epicotyl and cotyledon during development reflect the physiological role of these tissues. The epicotyl is not a part of the plant's storage system and would not be expected to show a rapid turnover of starch. In accordance, both enzymes were low in this tissue throughout the time period investigated. The cotyledons, which supply reserve food to the growing plant, age and die after an initial period of metabolic activity. Accordingly, the enzyme levels remained relatively constant or decreased, and after the seventeenth day neither activity could be detected.

Table VI. *Specific Activities of Hydroxypyruvate:L-Alanine and Phosphohydroxypyruvate:L-Glutamate Transaminases During Pea Development*

Experimental conditions as described in table I.

Tissue	Age	Hydroxypyruvate : L-alanine transaminase	P-Hydroxypyruvate : L-glutamate transaminase
	days	*mμmoles per mg protein per min*	
Seed		<0.1	1.8 ± 0[1]
Leaf	10	8.2 ± 0.5	4.6 ± 0.1
"	11	8.5 ± 1.6	4.7 ± 0.4
"	13	8.8 ± 1.6	4.2 ± 0.2
"	15	13.1 ± 0.2	4.5 ± 0.6
"	16	11.1 ± 0.7	4.0 ± 0.6
Apical meristem	10	9.4 ± 0.6	8.7 ± 0.7
"	11	9.5 ± 1.1	11.1 ± 0.4
"	13	8.6 ± 1.1	10.7 ± 0.4
"	15	8.5 ± 0.6	12.4 ± 0.8
"	16	8.3 ± 0.9	11.7 ± 0.3

[1] Mean deviation of results from 2 separate growth experiments.

Developmental Studies on Transaminases. The levels of activity of hydroxypyruvate:L-alanine and P-hydroxypyruvate:L-glutamate transaminases found during development of the pea plant are given in table VI. Because of the amounts of enzyme preparation required for the transaminase assays, only

Table VII. *Assay for* D-*Glycerate Kinase in Pea Leaves*

Pea leaves from plants grown in the field were treated and assayed as described under Materials and Methods.

Components of assay system	Phosphoglycerate formation
	mμmoles per mg protein per min
Complete	15.0
Complete	15.5
Minus D-glycerate	0
Minus ATP	0
Minus enzyme	0

seed, leaf and apical meristem were investigated. In contrast to the dehydrogenases, the activities of both transaminases remained relatively constant in both leaf and apical meristem over the time period studied. However, the levels of activity of a given transaminase are generally comparable to those of the dehydrogenase of the corresponding pathway in the tissues investigated. In seeds, for example, hydroxypyruvate:L-alanine transaminase is barely detectable, as is GDH, whereas the 2 enzymes of the phosphorylated pathway are readily demonstrable. In leaf, the activity of hydroxypyruvate:L-alanine transaminase is higher than that of P-hydroxypyruvate:L-glutamate transaminase in agreement with the relative levels of activity of the 2 dehydrogenases in this tissue. Although there is no decrease in the level of activity of hydroxypyruvate:L-alanine transaminase in apical meristem as compared to leaf (as was observed with GDH), the activity of P-hydroxypyruvate:L-glutamate transaminase is higher in this tissue than it is in leaf which is consistent with the changes observed for PGDH in these 2 tissues.

In view of the relationship of D-glycerate to glycolytic intermediates and of the high levels of activity of the enzymes of the nonphosphorylated pathway in leaves, studies were carried out to determine if glycerate kinase is present in pea leaves. The results (table VII) clearly establish the presence of this enzyme in this tissue.

Discussion

The main purpose of this study was to gain insight into the relative significance of the phosphorylated and nonphosphorylated pathways for serine metabolism during development. Since germinating seed consists of an organized collection of many tissues which differ widely in function and fate, it was necessary to investigate the individual parts of the developing plant if meaningful results were to be obtained. On the basis of the relative levels of activity of the individual enzymes, the present studies indicate that the phosphorylated pathway is of major importance for serine metabolism in tissues associated with rapid cell proliferation, *e.g.* seed and apical meristem, and that the nonphosphorylated pathway plays a predominant role in green leaves. The functional role of both pathways must be closely associated with carbohydrate metabolism in view of the intermediates involved.

In general, the routes for carbohydrate utilization or formation in plants vary not only with the stage of development but also with different types of plants. Since the pea is a starch-storing seed, upon germination there is a rapid turnover of this material as evidenced by respiratory quotients of approximately one. Although starch is the major reserve in the endosperm, fat and protein also play an important role in seed development. As germination proceeds, the plant begins to replenish its sugars concomitant with the onset of photosynthesis. In green tissues, hexose phosphates are the net products of carbon assimilation by the photosynthetic carbon cycle. Another route for sugar formation involves the glyoxylate cycle in which a net conversion of carbon from fatty acids to carbohydrate is effected. This pathway is known to be of importance in fat-storing seeds during germination when massive breakdown of stored fat and its conversion to carbohydrate occurs (2). The key enzymes of this pathway, *i.e.* isocitritase and malate synthetase, are known to be absent from seed until germination when *de novo* synthesis occurs (12, 17). A third route by which plants can form precursors of hexose is the glycolate, or glyoxylate-serine, pathway. D-Glycerate has been demonstrated as an intermediate in the pathway from glycine or serine to sucrose in pea leaves (15).

The enzymes of the nonphosphorylated pathway, GDH and hydroxypyruvate:L-alanine transaminase, must function in the glycolate pathway and thus provide the enzymatic link between serine and D-glycerate. The role of these 2 enzymes in this gluconeogenic route is further supported by the present demonstration of a D-glycerate kinase in pea leaves. This activity was shown to require D-glycerate and to be dependent upon ATP. The presence of this kinase completes the circuit of enzymes necessary for the conversion of serine to triose-P precursors of sugars. That these enzymes are involved in a gluconeogenic role in leaf is further indicated by the results of the light *vs.* dark growth experiments. When peas were grown in the dark, leaves were found to contain reduced levels of GDH over those present in light-grown plants; a similar result was obtained with apical meristem. These observations correlate well with the findings of Miflin *et al.*, (15) who showed that in pea leaves the conversion of serine to sucrose is light dependent.

The present studies do not rule out the possibility that in certain tissues, *e.g.* leaf, apical meristem, the nonphosphorylated pathway may also serve as a route for serine biosynthesis from carbohydrate intermediates. In fact, the labeling patterns in D-glycerate, formed in tobacco leaves during $^{14}CO_2$ photosynthesis, suggest that this compound can be formed *in vivo* directly from intermediates in the reductive pentose phosphate cycle as well as *via* the glycolate

pathway (6). By analogy to animal systems, D-glycerate could arise either from D-2-P-glycerate by the action of a specific phosphatase (4) or more indirectly from fructose by the following conversions: fructose → fructose-1-P → D-glyceraldehyde → D-glycerate (10). In plant tissues, sucrose would provide a ready source of fructose by the action of invertase (sucrase).

The fact that ungerminated seeds contain significant levels of PGDH and P-hydroxypyruvate:L-glutamate transaminase, while those of GDH and hydroxypyruvate:L-alanine transaminase are either absent or very low, is indicative that the phosphorylated pathway is utilized for serine formation in this tissue. In addition, the results suggest that in those tissues associated with rapid cell proliferation, such as apical meristem, the same pathway is of greater importance. Since D-3-P-glycerate is one of the early products of CO_2 fixation in the photosynthetic carbon cycle, the first substrate for the phosphorylated pathway is readily available in tissues carrying out photosynthesis. Although the levels of activity of GDH and hydroxypyruvate:L-alanine transaminase exceed those of PGDH and P-hydroxypyruvate:L-glutamate transaminase in leaf, the latter enzymes are readily demonstrable in this tissue. These results are consistent with those from *in vivo* $^{14}CO_2$ studies which indicate that both cytoplasmic (glycolate pathway) and chloroplastic (phosphorylated pathway) routes function for serine biosynthesis in plant leaves (*cf.* 6).

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