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Alanine Synthesis by Bundle Sheath Cells of Maize[1]

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ABSTRACT

Because in the phloem sap of maize (*Zea mays* L.) leaves a quarter of the total amino nitrogen can be found as alanine, the capacity of a *de novo* synthesis of alanine from 3-phosphoglycerate (3-PGA) was studied with isolated bundle sheath (BS) strands of maize. Inasmuch as these cells have retained their plasmodesmatic openings, it was possible to study the formation of alanine from 3-PGA when glutamate and ADP were being added. Alanine synthesis required the existence of the intact cell structure. From the formation of the intermediates, partially released to the medium, the activities of the enzymes of the reaction chain from 3-PGA to alanine could be measured in the intact cells. The results show that in the BS cells the rate of alanine production from pyruvate (0.5 micromole/minute per milligram BS chlorophyll) is more than sufficient to produce one-fourth of the assimilated nitrogen as alanine. As the activity of pyruvate kinase in intact bundle sheath cells in the light was found to be only 0.2 micromole/minute per milligram BS chlorophyll, it is concluded that in the light part of the conversion of 3-PGA to pyruvate may not occur via pyruvate kinase reaction, but via phospho*enol*pyruvate carboxylase, NADP-malate dehydrogenase, and NADP-malic enzyme in the mesophyll and BS cells.

In C_4 plants such as maize the mechanical disruption of leaves by means of a fast rotating knife results in a preferential disruption of the mesophyll cells (3). As a result of this, BS[2] strands can be obtained consisting of a segment of vascular bundle surrounded by BS cells of high functional integrity and metabolic competence (3, 4). In these cells the plasmodesmata that originally connected the BS cytosol with mesophyll cells (21) are retained in their entirety (2, 25, 26). Due to the plasmodesmatic connections the BS cells of the isolated BS strands are permeable for molecules up to a molecular mass of about 900 D (2, 25). This accessibility of highly intact cells to substrates added to the outside medium makes BS cells very interesting for metabolic studies.

Studies conducted in our laboratory have shown that in the phloem sap of maize leaves 28% of the amino nitrogen occurred as alanine, 28% as glutamine, 13% as glutamate, and 13% as asparagine (27). These results indicated that about a quarter of the assimilated nitrate is exported as alanine. A *de novo* synthesis of alanine from 3-PGA and glutamate may be expected to take place in the BS cells, because 3-PGA is formed there by photosynthesis. Moreover, previous studies on photosynthetic induction in leaves of *Zea mays* (8, 24) indicated that in the BS cells the glycolytic pathway from 3-PGA to pyruvate is operating and glutamate is found in BS cells at high concentrations (19). In the present report we investigated the capacity of BS cells to produce alanine from 3-PGA.

MATERIALS AND METHODS

Plant Growth

Zea mays was grown in soil under natural illumination supplemented with incandescent and cool white fluorescent lamps to provide 400 to 700 $\mu E \cdot m^{-2} s^{-1}$ in a glasshouse at 25 to 30/15 to 18°C day/night temperature.

Preparation of BS Strands

BS strands were isolated from fully expanded leaves of 3 to 4 week old plants by blending 2 mm deribbed leaf segments in a polytron giving 1 s (setting at 7) and 15 s (setting at 5–6) bursts. The blending medium contained 0.3 M sorbitol, 20 mM Hepes-KOH (pH 7.6), 4 mM $MgCl_2$, 2 mM Pi, and 10 mM isoascorbic acid. The crude homogenate was filtered through 1.3 and 1 mm sieves and 600 μm and 280 μm nylon nets. The BS strands were collected by filtration on an 88 μm nylon mesh, washed, resuspended on 100 mL of resuspending medium (0.3 M sorbitol, 20 mM Tricine-KOH (pH 8), 4 mM $MgCl_2$, 2 mM Pi, and 10 mM KCl) and filtered again. Finally, the BS strands were washed off from the mesh, allowed to settle, resuspended in approximately 50 μg Chl per mL resuspending medium and stored on ice.

Determination of Metabolite Synthesis by Intact BS Cells

Intact BS strands (30–50 μg Chl/mL) suspended in resuspending medium (see above) were incubated for 2 min with the additions indicated (50 mM KCl was included when assaying pyruvate kinase and PEP-phosphatase) and reactions were started by adding the corresponding substrate at 30°C either in darkness or with illumination by a slide projector giving an incident irradiance of approximately 1200 $\mu E/m^2 \cdot s$. At different times, reactions were stopped by 3% $HClO_4$, neutralized by KOH, and metabolite concentrations were spectrophotometrically measured in a coupled assay and in the following sequence: pyruvate with lactate dehydrogenase, PEP with pyruvate kinase, 2-PGA with enolase, and 3-PGA with PGA mutase as described by Lamprecht and Heinz (18).

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[2] Abbreviations: BS, bundle sheath; DHAP, dihydroxyacetonephosphate; PEP, phospho*enol*pyruvate; PGA, phosphoglycerate.

Alanine was also assayed enzymatically according to Grassl and Supp (9).

Mesophyll Protoplast Preparation

About 15 g of leaves were sliced into 1 mm segments and digested 2.5 h (30°C, under illumination) in 100 mL of medium containing 1% Sumizyme cellulase (from *Trichoderma viride*, a generous gift from Dr. J. Onishi) and 0.05% pectolyase Y-23, 0.5 M sorbitol, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Mes-KOH (pH 5.5), 20 mM L-ascorbate, and 0.1% BSA. Digested leaf segments were resuspended in sucrose medium (0.5 M sucrose, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM Hepes-KOH (pH 7.6), and 0.1% BSA). Segments were gently agitated until well dispersed, then filtered through a tea strainer and 80 μm nylon net. The filtrate was partitioned into glass tubes, overlaid successively by sucrose-sorbitol and sorbitol media (like sucrose medium but containing 0.4 M sucrose + 0.1 M sorbitol and 0.5 M sorbitol, respectively) and centrifuged at 300*g* for 10 min (4°C). The interphase between sucrose-sorbitol and sorbitol media contained only mesophyll protoplasts and was diluted two- to threefold with KCl medium (5 mM Hepes-KOH (pH 7.6), 250 mM KCl, 1 mM $CaCl_2$, and 0.2% BSA) and centrifuged at 200*g* for 5 min. The pellet containing mesophyll protoplasts was resuspended as described below.

Preparation of Extracts

Leaf and BS strand extracts were prepared by grinding 1 to 2 g of leaves or BS strands (200 μg Chl) in a mortar and pestle with 2 mL extraction buffer (50 mL Hepes-KOH [pH 7.2], 10 mM DTT, 1 mM EDTA, and 0.1% [w/v] PVP-40), filtering the homogenate through Miracloth, and centrifuging the filtrate for 30 s in an Eppendorf Microfuge. All operations were conducted between 0 and 4°C. Mesophyll protoplast extracts were prepared by resuspending the cells into extraction buffer plus 0.1% Triton X-100 and centrifuging as described above.

Enzyme Assays

Alanine aminotransferase (10), PEP carboxylase (13), NADP-malate dehydrogenase (14), and NADP-malic enzyme (15) were assayed at 25°C as previously reported.

Chl Determination

BS strand suspensions were centrifuged, and the supernatant was removed and methanol added. The mixture was shaken several times for 1 h while it was protected from light. Chl was measured in the clear supernatant using the procedure of Wintermans and DeMots (28).

RESULTS AND DISCUSSION

Distribution of Enzymes

To determine the distribution of enzymes involved in the synthesis of alanine in maize leaves, the activities of these enzymes were assayed in extracts from whole maize leaves and from isolated BS cells and mesophyll protoplasts. The results shown in Table I are average values obtained from various samples. The activity values are related to Chl content of the leaves and the BS cells and mesophyll cells, respectively. It may be noted that the respective Chl contribution of each cell type to the total leaf Chl determined algebraically from the Chl *a*/Chl *b* ratios (29) was 70% in the mesophyll cells and 30% in the BS cells. This figure may be variable according to growth conditions, because the Chl distribution between mesophyll and BS cells in maize was found in other studies to be 64:36 (16) and 80:20 (8). To relate the activities found in the mesophyll and BS cell preparation to those in the leaves, the activities have been also expressed as percentage of the total activity found in leaf extracts. These values are shown in Table I in parentheses.

In the case of the BS marker enzyme NADP-malic enzyme and the mesophyll marker enzymes PEP-carboxylase and NADP-malate dehydrogenase the enzyme distribution found is according to the expectation, indicating that the mesophyll and BS cell preparations were not markedly contaminated by each other and that the values for the Chl contents of both cell preparations, to which the enzyme specific activities have been related, are in the same range as estimated from the Chl *a*/Chl *b* ratios. For some enzyme activities, the percentage values of the BS and mesophyll enzyme activities do not add up to 100. There may be two reasons for this. As the activity measurements, of which average data are shown in Table I, have been performed with different cell preparations and different leaf samples over a longer time span, considerable fluctuations occurred of enzyme activities in whole leaves and in the two cell types. Furthermore, the activities of other leaf cells apart from mesophyll and BS cells, in particular epidermal cells, have not been determined.

As shown in Table I, the enzymes required for the interconversion of 3-PGA to alanine are all present in the BS and in the mesophyll cells, in agreement with earlier measurements (5, 8, 11, 17, 30), although in our measurements we found in

Table I. *Activities of Enzymes Involved in Reaction Sequence from 3-PGA to Alanine in Extracts of Maize Leaves and from Preparations of Mesophyll Cell and BS Strands from Maize Leaves*

Enzyme activities were measured as described in "Materials and Methods." Values in parentheses are percentages of enzymes present in each cell according to the percentage of Chl distribution in the mesophyll and BS cells. Mean values of at least four experiments.

Enzyme	Enzyme Activity		
	Leaf	Mesophyll	BS
		μmol min^{-1} mg Chl^{-1} (% of total)	
Phosphoglycerate mutase	3.16	2.95 (65)	2.69 (26)
Enolase	1.07	0.90 (59)	1.34 (38)
Pyruvate kinase	0.91	0.24 (18)	0.58 (19)
PEP phosphatase	1.26	0.94 (52)	0.41 (10)
Alanine aminotransferase	1.02	0.37 (25)	2.44 (72)
PEP carboxylase	14.98	21.40 (100)	0.36 (0)
NADP-malic enzyme	11.61	0.34 (0)	37.45 (97)
NADP-malate dehydrogenase	9.03	11.88 (93)	0 (0)
Chl *a*/*b*	4.22	3.49	7.34
% Chl	100	70	30

BS cells higher activities of phosphoglyceromutase and enolase than earlier reported (8, 17). This may be due to different cultivars and different growth conditions. We found considerable differences in the activities of alanine aminotransferase activity in leaf extracts of different cultivars. Earlier measurements of pyruvate kinase had shown that the major portion of the enzyme is located in the epidermal cells (30). This explains why in our measurements only about half of the total leaf activity can be attributed to the mesophyll and BS cells. Earlier measurements of pyruvate kinase had not differentiated between pyruvate kinase and PEP-phosphatase activity. Our results show that pyruvate kinase activity is about equally distributed between the mesophyll and BS cells, whereas most of the PEP-phosphatase is located in the mesophyll cells.

Alanine Synthesis by BS Strands

In the experiment of Figure 1A, BS strands were suspended in a medium containing ADP and glutamate. Upon the addition of 3-PGA (start of the reaction) a rapid formation of 2-PGA, PEP, pyruvate, and alanine is observed. From the initial linear slope, the rates of the formation of the various metabolites can be determined. Figure 1B shows a parallel experiment, in which the samples obtained after the start of incubation were rapidly centrifuged to sediment the BS cells for assay of metabolites in the supernatant only. The results show that a major part of the formed metabolites were released into the medium, which was to be expected in view of the open plasmodesmatic connections. In another parallel experiment (not shown in the figure) the BS cells were centrifuged prior to the experiment, and the resultant supernatant of the BS cell suspension was incubated. The formation of 2-PGA (0.02 μmol/min·mg Chl) was very low and the subsequent products PEP, pyruvate, and alanine could not be detected at all. These results clearly demonstrate that the supernatant was almost free of BS enzyme. The formation of alanine requires the existence of the intact cell structure. In the experiment of Figure 2, where the BS strands were disrupted prior to the incubation, there was also a rapid formation of 2-PGA and PEP, but the rate of pyruvate formation was extremely low, and no alanine was detected. In the extract, the intermediate concentrations were apparently too low for alanine formation. In Table II the results of a series of experiments are shown in which the components of the incubation of BS cells were varied. The experiments in Figure 1 and in part of Table II were carried out with a 3-PGA concentration of 10 mM, which is near the 3-PGA concentration found in the light in BS cells of intact maize leaves (13 mM) (22). In Table II the experiment was also carried out with 1 mM 3-PGA, similar to the concentration found in maize leaf BS cells in the dark (1.5 mM) (22). Under these conditions, the rate of alanine synthesis was still about 60% of the rate obtained with 10 mM 3-PGA. For the synthesis of alanine, 3-PGA could be replaced by 2-PGA or PEP. When glutamate was omitted, 2-PGA, PEP, and pyruvate were also produced, as to be expected, but no alanine synthesis was observed. When 5 mM aspartate was added instead of glutamate, no alanine was formed (data not shown). Thus, aspartate could not replace glutamate as amino nitrogen donor, reflecting the specificity of alanine aminotransferase (8). When ADP was omitted, with 3-PGA or 2-PGA as

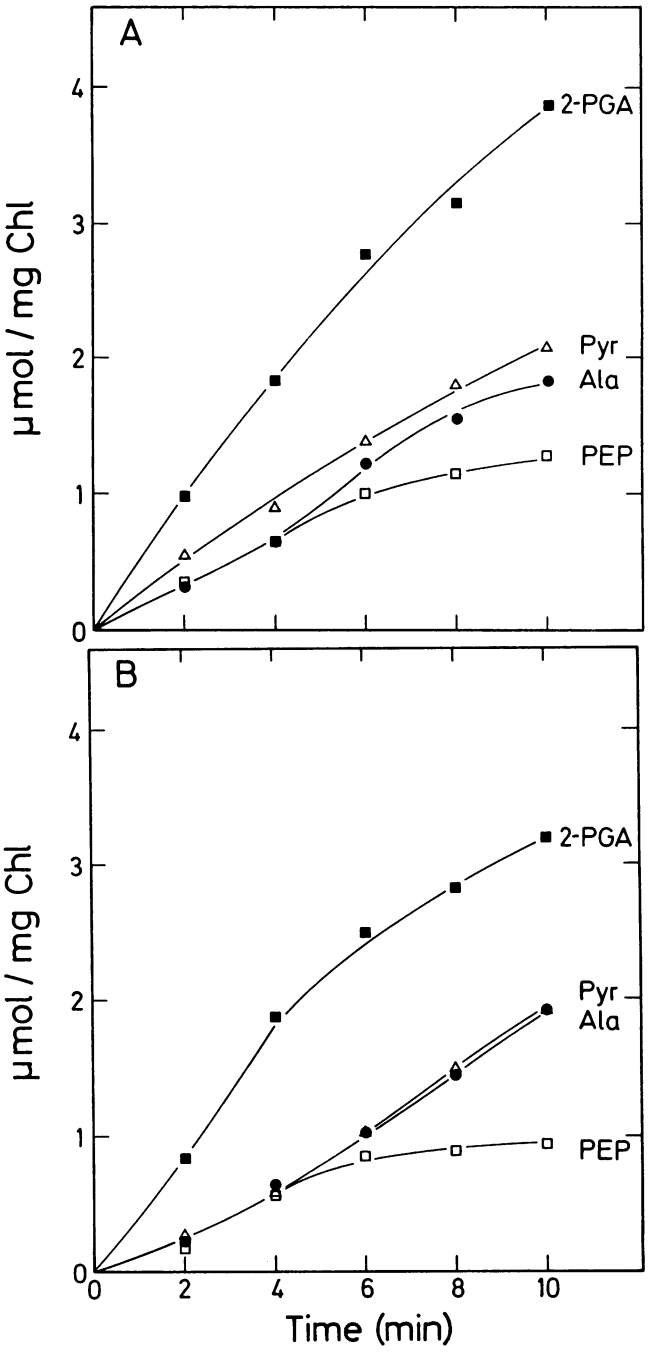

Figure 1. Synthesis of alanine from 3-PGA by BS strands. BS strands were incubated with 1 mM ADP, 5 mM glutamate, and the reaction was started by adding 10 mM 3-PGA. Other additions and metabolites concentrations were according to "Materials and Methods." (A) Metabolites in whole suspension of BS strands. (B) Metabolites in the supernatant of the suspension of BS strands obtained by centrifugation after the end of incubation.

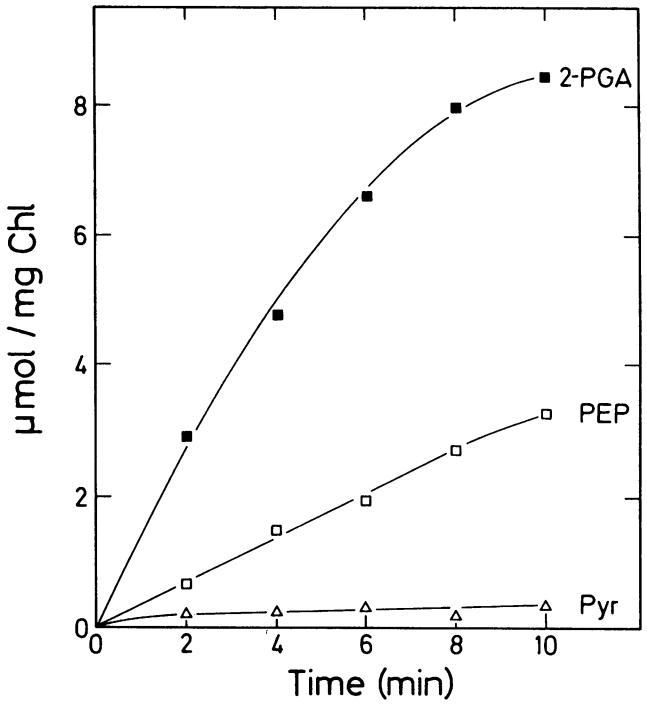

Figure 2. Synthesis of alanine from 3-PGA in an extract of BS strands. The BS suspension of the experiment in Figure 1 was disrupted prior to the incubation by grinding in a mortar and then incubated as in the experiment of Figure 1.

substrate, the formation of pyruvate was decreased and the production of alanine could not be detected. With PEP as substrate the remaining pyruvate synthesis in the absence of ADP and glutamate was enhanced. The question arose whether the formation of pyruvate and alanine in the absence of ADP was due to the activity of PEP-phosphatase. For this the conversion of 1 mM PEP by BS cells was measured in the presence and absence of ADP at various pH values according to the experiment of Table II. As shown in Figure 3, the rate of PEP conversion in the absence of ADP, designated as PEP-phosphatase activity, strongly decreased from pH 6.8 to 8, resembling the pH dependence of PEP-phosphatase with optimal activity at pH 5.5 (6). On the assumption that the activity of PEP phosphatase determined in the absence of ADP is also present in the presence of ADP, the rate of pyruvate kinase might be determined from the rate of PEP conversion in the presence of ADP subtracted by the rate obtained in the absence of ADP. The pH dependence of pyruvate kinase activity obtained in this way shows a broad optimum from pH 7.2 to 8.4. Broad profiles centered between pH 7 and 8 have been reported for pyruvate kinase from spinach, castor bean, and pea leaves (1, 12) and appear to be characteristic of the cytoplasmic isoenzyme. Because the plastid isoenzyme exhibits a narrow optimum at pH 8 (12), we are probably measuring an overlapping of the pH dependence of both isoenzymes. These results allow the conclusion that in the BS cells pyruvate kinase and also PEP phosphatase are operating under physiological conditions.

In the experiment of Table III, BS cells were incubated under simulated light conditions in a medium containing DHAP and 3-PGA in a ratio 2:10, glutamate, and ADP. When malate was also added, there was a large increase in the rate of pyruvate production, reflecting the activity of malic enzyme, and alanine synthesis increased as well. In the control experiment carried out in the dark, where the conversion of malate into pyruvate is very low (see also ref. 4), malate has no marked stimulatory effect on alanine synthesis.

Determination of Enzyme Activities Participating in Alanine Synthesis in Intact BS Cells

The results of Table II make it possible to determine the rate at which each single enzyme of the metabolic pathway from 3-PGA to alanine is operating in the intact BS cells under the conditions set by the additions of metabolites from outside. Thus the rate of 3-PGA conversion catalyzed by phosphoglyceromutase can be determined from the sum of the rates of the formation of 2-PGA, PEP, pyruvate, and alanine. Enolase activity can be evaluated from the rate of PEP + pyruvate + alanine formation. The sum of pyruvate kinase and PEP-phosphatase activities is evaluated from the rate of pyruvate + alanine formation in the presence of ADP. The corresponding activity in the absence of ADP reflects PEP phosphatase activity. On the assumption that ADP does not alter PEP-phosphatase activity, pyruvate kinase activity is determined from the difference of activities in the presence and the absence of ADP. The value for alanine aminotrans-

Table II. *Rates of Formation of Metabolites by BS Strands from Maize Leaves in the Dark*

Reaction was started by the first metabolite listed in the "Additions." Other assay conditions were as described in "Materials and Methods."

Additions (mM)	2-PGA	PEP	Pyr	Ala
		µmol min⁻¹ mg Chl⁻¹		
3-PGA (1), 2,3-PGA (0.05), ADP (1), Glu (0.5)	0.12	0.05	0.19	0.11
3-PGA (10), 2,3-PGA (0.05), ADP (1), Glu (5)	0.38	0.13	0.21	0.19
3-PGA (10), 2,3-PGA (0.05)	0.55	0.27	0.10	0
2-PGA (2), ADP (1), Glu (5)		0.12	0.08	0.23
2-PGA (2)		0.23	0.09	0
PEP (1), ADP (1), Glu (5)			0.63	0.30
PEP (1), Glu (5)			0.20	0.21
PEP (1), ADP (1)			1.12	0
PEP (1)			0.32	0

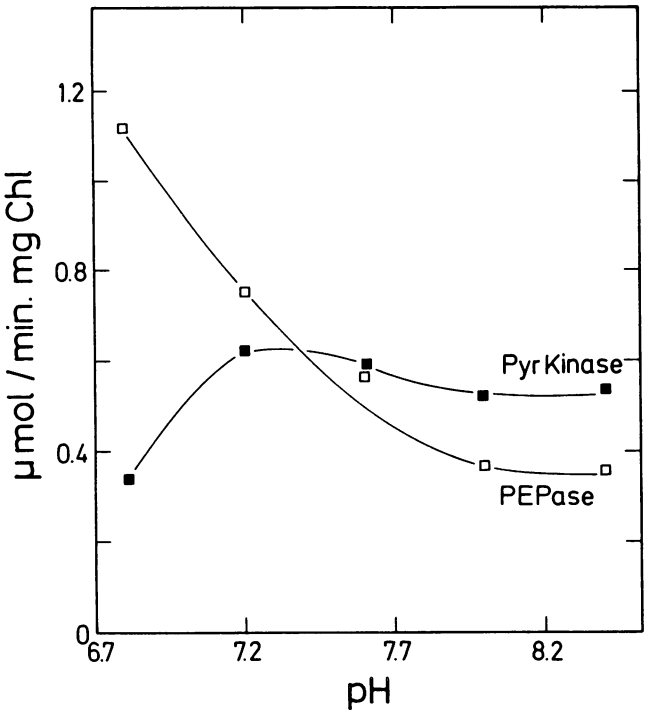

Figure 3. pH dependence of pyruvate kinase and PEP-phosphatase in intact BS cells. BS strands were suspended in 50 mM Hepes-50 mM Tricine, 0.3 M sorbitol, 12 mM $MgCl_2$, 50 mM KCl, and 1 mM ADP, incubated 2 min at 30°C; the reaction was started by adding 1 mM PEP. The reaction was stopped and pyruvate was measured as described in "Materials and Methods." PEPase, pyruvate synthesized without ADP in the reaction media; PyrKinase, pyruvate synthesized in the presence of ADP minus PEPase.

ferase activity can be taken directly from the rate of alanine formation in Table II. Likewise, the activities of the above-mentioned enzymes have also been evaluated from the results in Table III. The results are shown in Table IV.

Comparison of Enzyme Activities Involved in Alanine Synthesis

Due to the open plasmodesmatic connections, allowing the uptake of substrates and the release of products, intact isolated BS cells are able to catalyze the conversion of 3-PGA to alanine. Although the intermediate products of the reaction chain, 2-PGA, PEP, and pyruvate, partially leak out into the medium, there appears to be no equilibration of the internal metabolite concentrations with those in the medium. It seems as if the internal metabolite concentrations are kept high enough for the pathway to operate with the possibility of surplus intermediates to be released to the medium. This opens the unique possibility to assay the activities of the different enzymes of a reaction chain in an intact cell (Table IV). It may be noted that for the incubation of BS strands physiological substrate concentrations have been employed. Under these conditions, for the enzymes phosphoglyceromutase, enolase, and pyruvate kinase, the activities measured in the functioning BS cells amounted to 30 to 40% of the activity assayed in the BS extract under saturating conditions. This shows that the isolated BS cells were indeed very active.

A comparison of enzyme activities shows that the activities of phosphoglyceromutase and enolase exceed pyruvate kinase activity. Besides pyruvate kinase there is also a minor activity of PEP-phosphatase observed, which could be elevated when 1 mM PEP was added to the BS cells. We found for PEP-phosphatase a variation of activities in various cell preparations—in some BS cell preparations it could not be detected at all. In plant cells, PEP-phosphatase was found to be induced by phosphate starvation, and it has been suggested that this enzyme is part of a bypass of phosphate and adenylate dependent glycolytic enzymes (7). The variations in activities observed in our BS cell preparations could be therefore the results in a variation of growth conditions or leaf age. As shown in Table IV, the sum of the activities of pyruvate kinase and PEP-phosphatase was decreased upon illumination. In a series of similar experiments in which there was differentiation between pyruvate kinase and PEP-phosphatase activity we found consistently that the metabolite flow through pyruvate kinase was reduced upon illumination. Average results from three experiments yielded that in intact BS cells pyruvate kinase activity decreased from 0.32 µmol/min·mg Chl in the dark to 0.19 in the light. Moreover, when pyruvate kinase was assayed in intact BS cells with PEP as substrate the enzyme activity was reduced in the light by 30%. The mechanism of this apparent decrease of activity in the light is not clear. It could be due to a control by adenylates (23) but we were not able to reverse the apparent inhibition in the light by uncoupler or to simulate this inhibition in the dark by addition of ATP (experiments not shown).

Nevertheless, these results indicate that particularly in the light the rate of alanine synthesis from 3-PGA via pyruvate kinase may be limited by the pyruvate kinase. This is supported by data in Table III where an increased production of pyruvate caused by the activity of malic enzyme in the light also results in an increased formation of alanine. But the increase in the rate of alanine formation by a factor of 2.5, as related to the dark experiments, is low in comparison to the increase in pyruvate production by a factor of about 15. Even under these favorable conditions, the rate of alanine formation (0.50 µmol/min·mg Chl) is only about 20% of alanine aminotransferase activity assayed in BS extracts (Table I). This result is in good agreement with earlier measurements

Table III. *Rates of Formation of Metabolites by BS Strands from Maize Leaves in the Light and in the Dark*

Incubation medium contained 1 mM ADP, 0.05 mM 2,3-bis-PGA, 5 mM glutamate, and 2 mM DHAP. The reaction was started by addition of 10 mM 3-PGA. Other assay conditions were as described in "Materials and Methods."

Conditions	Additions	2-PGA	PEP	Pyr	Ala
		µmol min⁻¹ mg Chl⁻¹			
Light	None	0.46	0.20	0.09	0.09
	Malate (10 mM)	0.64	0.26	4.60	0.50
Dark	None	0.48	0.10	0.17	0.12
	Malate (10 mM)	0.60	0.11	0.31	0.18

Table IV. *Determination of Enzyme Activities in BS Strands by Performing Alanine Synthesis from Data of Tables II and III*

Experiment	Additions (mM)	Phospho-glycero-mutase	Enolase	Pyruvate Kinase + PEPase[a]	PEPase	Pyruvate Kinase
			μmol/min⁻¹ mg Chl⁻¹			
A. Dark	3-PGA (1), 2,3-PGA (0.05), ADP (1), Glu (0.5)	0.47	0.35	0.30		
	3-PGA (10), 2,3-PGA (0.05), ADP (1), Glu (5)	0.91	0.53	0.40		0.30[b]
	3-PGA (10), 2,3-PGA (0.05)	0.92	0.37		0.10	
	2-PGA (2), ADP (1), Glu (5)		0.43	0.31		0.22[b]
	2-PGA (2)		0.32		0.09	
	PEP (1), ADP (1), Glu (5)			0.93		0.52[b]
	PEP (1), Glu (5)				0.41	
B. Light	DHAP (2), 3-PGA (10), ADP (1), Glu (5)	0.84	0.38	0.18		
Dark	DHAP (2), 3-PGA (10), ADP (1), Glu (5)	0.87	0.39	0.29		

[a] PEPase, PEP-phosphatase. [b] Values obtained by subtraction of PEPase activity assayed in the absence of ADP.

by Chapman *et al.* (5) who found in BS extracts from maize an alanine aminotransferase activity of 2.6 μmol/mg Chl·min and a maximal activity for the conversion of pyruvate + glutamate to alanine + 2-oxoglutarate by BS cells of 0.65 μmol/mg Chl·h. These authors also have shown that in maize leaves BS cells alanine aminotransferase activity is largely or completely located in the mitochondria (5).

CONCLUDING REMARKS

In the present study BS cells have been used as a model for studying the capacity of these cells to produce pyruvate and alanine. In agreement with earlier results, BS cells were found to have the capacity of producing alanine from pyruvate and glutamate at a rate of 0.5 μmol/min·mg BS Chl. Translated into total leaf Chl this is equivalent to a rate of 7.5 μmol/h·mg total Chl. Assuming an N:C assimilation ratio of 1:20, and a rate of CO_2 fixation of 200 μmol/h·mg total Chl this rate is more than sufficient to produce one-fourth of the assimilated nitrogen as alanine, that amount found in the phloem. In the case of pyruvate kinase, on the other hand, the activity in illuminated BS cells (0.2 μmol/min·mg Chl equal to 3 μmol/h·mg total Chl) does not seem to be sufficient for supplying the carbon skeleton required for alanine as well as for glutamate and glutamine as major nitrogen assimilation products. It seems therefore that in the light the major route of the conversion of 3-PGA to pyruvate is not via pyruvate kinase reaction, but via phosphoglyceromutase, enolase, PEP-carboxylase, NADP-malate dehydrogenase, and NADP-malic enzyme.

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