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# Photosynthetic carbon reduction pathway is absent in chloroplasts of *Vicia faba* guard cells

(stomata/Calvin–Benson cycle/palisade parenchyma/spongy parenchyma/transpiration)

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**ABSTRACT** **Four cell types from *Vicia faba* Linnaeus 'Long Pod' leaflets were assayed for three enzymes unique to the photosynthetic carbon reduction pathway. The enzymes were ribulosebisphosphate carboxylase [3-phospho-D-glycerate carboxy-lyase (dimerizing), EC 4.1.1.39], phosphoribulokinase (ATP:D-ribulose-5-phosphate 1-phosphotransferase, EC 2.7.1.19), and glyceraldehyde-phosphate dehydrogenase ($NADP^+$) (phosphorylating) [D-glyceraldehyde-3-phosphate:$NADP^+$ oxidoreductase (phosphorylating), EC 1.2.1.13]. On a dry weight basis, these enzyme activities were about twice as high in palisade as in spongy parenchyma. Two of the enzymes were not detected in epidermal cells and the other was present in only a trace amount. In guard cells, these enzyme activities were absent or present at less than 1% of the amount in palisade cells. Immunoelectrophoresis showed that ribulosebisphosphate carboxylase was absent in extracts of guard cell protoplasts. Microscopy confirmed the abundance of typical guard cell chloroplasts. These results demonstrate the absence of the photosynthetic carbon reduction pathway in guard cell chloroplasts. This is the only chloroplast type known to be deficient in this pathway in plants whose primary $CO_2$ acceptor is ribulose bisphosphate. Possible reasons for the absence of this pathway in guard cells are discussed.**

Gas exchange between a leaf and the atmosphere is almost exclusively through stomata in the epidermis. Stomatal aperture is varied to minimize $H_2O$ loss while admitting $CO_2$. The physical basis for opening is swelling of the surrounding guard cell pair in response to the large negative osmotic potential resulting from $K^+$ influx (1). Potassium uptake is electrically balanced by $Cl^-$ uptake (2) and the synthesis of organic anions from starch (3–6).

An important issue in understanding stomatal physiology is the degree to which the guard cells are autotropic. Generally, guard cells contain chloroplasts, but epidermal cells have no or only a few rudimentary plastids. In *Vicia faba*, the percentage of cell volume occupied by chloroplasts is the same in guard cells as in palisade cells (7), the major photosynthetic cell type. Except for those in mesophyll cells of the plants that fix $CO_2$ into $C_4$ dicarboxylic acids (8), all chloroplasts studied contain the photosynthetic carbon reduction pathway. Previous reports on $CO_2$ reduction in guard cells have conflicted, owing to the small size of a pair of guard cells (≈8 pl) and their interspersion with other tissue (guard cells occupy about 5% of epidermal volume). We investigated this question by using histochemical techniques to compare the activity of ribulosebisphosphate ($RuP_2$) carboxylase in guard cells to that in other leaf cells. The assay used for guard cells would have detected the activity in two or three palisade cell chloroplasts. In addition, extracts of guard cell protoplasts were analyzed by an immunological technique for $RuP_2$ carboxylase. To confirm the results, the activities of two other enzymes that are also unique to the photosynthetic carbon reduction pathway were estimated in guard cells and other leaf cells. Ultrastructures of chloroplasts in palisade and guard cells were compared.

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## MATERIALS AND METHODS

### Materials

Except as noted in the text, *Vicia faba* Linnaeus 'Long Pod' was used in all experiments. Plants were grown as described by Jones *et al.* (9) or as by Outlaw and Manchester (6).

Analytical enzymes were from Boehringer except $RuP_2$ carboxylase and ribose-*P* isomerase, which were from Sigma. Agarose was from Miles; most other chemicals were from Sigma.

### Microscopy

Guard cells in epidermal peels were examined by brightfield and fluorescence microscopy. Filters for fluorescence studies were Leitz BG12 and K580. For transmission electron microscopy, tissue was fixed at room temperature for 2 hr under partial vacuum in 100 mM Na cacodylate (pH 7) containing 3% (wt/wt) glutaraldehyde. The tissue was postfixed for 2 hr with 1% $OsO_4$ in the above buffer and then dehydrated in a graded ethanol series. The alcohol was replaced by propylene oxide before the tissue was embedded in Spurr's resin (10). Thin sections were stained with uranyl acetate and lead citrate (11) and then were examined in a Hitachi HU-11C electron microscope operated at 75 kV.

### Enzyme assays

Leaflets were illuminated (200 microeinsteins $m^{-2}$ $s^{-1}$ of 400- to 700-nm radiation) for at least 15 min before quenching in liquid $N_2$ that had been reduced to its freezing point by evacuation. [Illumination was to activate the enzymes (12–14).] Leaflets were then freeze-dried at −35°C. Freeze-drying did not change the activities of any of the enzymes reported here. Samples were dissected and weighed (5–15 ng) on a quartz fiber balance in a room with controlled temperature and humidity. The early analytical steps were conducted in a small droplet under oil to prevent evaporation. All enzyme activities were measured at 23°C, with the reaction initiated by addition of the tissue sample through the oil. Acid was used to destroy NAD(P)H before enzymatic amplification of the oxidized pyridine nucleotide (NAD, ref. 15; NADP, ref. 16). Tissue blanks and standards were carried through all steps. Except as noted, enzyme specific activities reported are based on dry weight. The concentration of most substrates was determined

Abbreviation: $RuP_2$, ribulose bisphosphate.

enzymatically by NADH extinction. Substrate concentrations in the specific steps were generally at about $6 \times K_m$. Details of histochemical techniques are given in Lowry and Passonneau (16).

**Ribulosebiphosphate Carboxylase [3-Phospho-D-glycerate Carboxy-Lyase (Dimerizing), EC 4.1.1.39].** *Specific step.* The reagent was 1 μl of 50 mM Tris·HCl (25 mM acid and 25 mM base) containing 10 mM $MgCl_2$, 1 mM reduced glutathione, 20 mM $KHCO_3$, 0.2 mM Ru*P*$_2$, and 0.02% bovine serum albumin. Standards contained 1, 2, 4, or 10 μM 3-*P*-glycerate. Tissue blanks contained no Ru*P*$_2$. This step was terminated after 15 min by the addition of the next reagent.

*Other analytical steps.* The 3-*P*-glycerate from the previous step was reduced by addition of 1 μl of 100 mM imidazole·HCl (75 mM acid and 25 mM base) containing 30 μM NADH, 0.1 mM EDTA, 0.3 mM ATP, 20 mM NaCl, 50 μg/ml of dialyzed glyceraldehyde-phosphate dehydrogenase ($NAD^+$)(phosphorylating) [D-glyceraldehyde-3-phosphate:$NAD^+$ oxidoreductase (phosphorylating), EC 1.2.1.12, from rabbit muscle], and 20 μg/ml of dialyzed 3-*P*-glycerate kinase (ATP:3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3, from yeast). This step (adapted from ref. 16) was terminated after 40 min by addition of 1 μl of 0.3 M HCl. A 1-μl aliquot was then transferred to 50 μl of enzymatic cycling reagent in a fluorometer tube with enzyme concentration set to provide about 1000 cycles in 1 hr, and then the assay was completed as usual (15).

*Comments on assay.* The procedure reported here differs in several respects from the procedures in the literature: (*i*) The assay was conducted by the addition of a whole freeze-dried cell and the enzyme was activated *in vivo* (12) instead of by preincubation with $Mg^{2+}$ and $CO_2$ (17), which simplified the assay. (*ii*) Ru*P*$_2$ carboxylase velocity was kept in the range of 10 μM $hr^{-1}$. In contrast, the assay of Bahr and Jensen (12), for example, resulted in a velocity of 1 mM $hr^{-1}$. (*iii*) 3-*P*-glycerate analysis was conducted in a second step at lower pH. This assay procedure was linear for 15 min as judged by a macroversion of the assay, using freshly prepared extracts (1-min time points). The microassay was also linear when palisade cells were assayed for 5, 10, and 15 min. Preincubation of palisade cells for 20 min with $Mg^{2+}$ and $CO_2$ [(Tris activation reagent of Grebanier *et al.* (18)] resulted in decreased activity. 3-*P*-glycerate reduction with the two-step method was complete as judged by NADH extinction [see Lilley and Walker (19) for details of this problem with pyridine-nucleotide-linked assays for this enzyme].

The pH shift and reagent dilution upon addition of the second reagent reduced the Ru*P*$_2$ carboxylase velocity to about 3% of that during the specific step; thus, the values reported here are marginally overestimated.

In some experiments, a 10-fold more sensitive procedure was used for guard cell pairs and epidermal cells. This was done by reducing the volumes of the specific step and 3-*P*-glycerate reduction step to 0.1 μl and increasing the enzyme composition in the cycling step by a factor of 5. (This more sensitive version of the assay would have easily detected 1/20th the activity present in a single palisade cell.)

To demonstrate that nothing present in guard cells interfered with the Ru*P*$_2$ carboxylase assay, whole leaf extract was made in 50 mM Tris·HCl (pH 8.1) and diluted to 20 mg (dry wt)/liter. Aliquots (0.5 μl) were taken and put under oil. A guard cell pair was added onto some of the droplets. Double-strength specific step reagent (0.5 μl) was added. All other analytical steps were carried out as usual. Whole leaf enzyme activity in the presence of a guard cell pair was indistinguishable from control values.

The procedure used to assay guard cell protoplast extracts (see *Immunoelectrophoresis*) for Ru*P*$_2$ carboxylase was like the first two steps of the microassay except that the volume was increased to 500 μl in each step (making oil well procedures unnecessary) and the assay was initiated by addition of 10 μl of extract (instead of by addition of single cells). Oxidation of NADH was determined fluorometrically.

**Phosphoribulokinase (ATP:D-Ribulose-5-Phosphate 1-Phosphotransferase, EC 2.7.1.19).** *Specific step.* The reagent was 1 μl of 50 mM Tris·HCl (25 mM acid and 25 mM base) containing 10 mM $MgCl_2$, 1 mM reduced glutathione, 20 mM $KHCO_3$, 3 mM ATP, 0.6 mM ribose-5-*P*, 0.02% bovine serum albumin, 5 μg/ml of ribose-5-*P* isomerase (D-ribose-5-phosphate ketol-isomerase, EC 5.3.1.6, from yeast), and 30 μg/ml of Ru*P*$_2$ carboxylase (from spinach). Standards contained 10, 20, or 50 μM Ru*P*$_2$. Tissue blanks contained no ribose-5-*P*. This step was terminated after 1 hr by heating to 80°C for 20 min.

*Other analytical steps.* The next reagent was 10 μl of 50 mM imidazole·HCl (25 mM acid and 25 mM base) containing 10 mM $MgCl_2$, 0.1 mM EDTA, 20 mM NaCl, 30 μM NADH, 25 μg/ml of dialyzed glyceraldehyde-*P* dehydrogenase ($NAD^+$) (phosphorylating), and 10 μg/ml of dialyzed 3-*P*-glycerate kinase. This step was terminated after 1 hr by addition of 1 μl of 2 M HCl. Amplification of $NAD^+$ was identical to that used for the Ru*P*$_2$ carboxylase assay.

*Comments on assay.* Ribulose-5-*P* was made in the assay reagent because the commercial product contained a contaminant that increased the blank values. The volume of the second step was much larger than the specific step volume in order to dilute substrate contaminants that inhibited the reduction of 3-*P*-glycerate. [The inhibition was similar to that found by Walbot (20).]

**Glyceraldehyde-Phosphate Dehydrogenase ($NADP^+$) (Phosphorylating) [D-Glyceraldehyde-3-Phosphate:$NADP^+$ Oxidoreductase (Phosphorylating), EC 1.2.1.13].** *Specific step.* The reagent was 2 μl of 100 mM Tris·HCl (67 mM acid and 33 mM base) containing 10 mM $MgCl_2$, 10 mM mercaptoethanol, 0.5 mM ATP, 50 μM NADPH, 2 mM 3-*P*-glycerate, 0.02% bovine serum albumin, and 3-*P*-glycerate kinase at 0.5 μg/ml. Standards contained 3, 5, or 8 μM $NADP^+$. Tissue blanks contained no 3-*P*-glycerate. This step was terminated after 1 hr by the addition of 1 μl of 0.3 M HCl.

*Other analytical steps.* A 1-μl aliquot was then transferred to 50 μl of enzymatic cycling reagent in a fluorometer tube with enzyme concentration set to provide about 1000 cycles in 1 hr and then the assay was completed as usual (16).

### Immunoelectrophoresis

The procedure is described in detail by Joseph (21) as adapted from Axelson *et al.* (22). Agarose (0.9%) containing 2% Ru*P*$_2$ carboxylase antibody [as pure IgG (23)] was poured onto glass plates. Extracts containing 60 ng of chlorophyll (24) of guard cell protoplasts (purified according to an unpublished procedure similar to that reported in ref. 25) were placed in 2.5-mm wells and subjected to electrophoresis at 4°C for 15 hrs (50 V per plate, Gelman Tris/barbital high resolution buffer). The plates were pressed, soaked twice in 100 mM NaCl, rinsed, and pressed again. The plates were stained with brilliant blue G (5.0 g/liter in 7 M aqueous ethanol containing 2 M acetic acid).

## RESULTS

### Structural observations

About 20 chloroplasts were in each *V. faba* guard cell pair. However, the guard cell chloroplasts were small and lacked an extensive internal membrane system. Their outline was round with some irregularities. Starch deposits were conspicuous.

Peripheral reticulum occupied a large part of the organelle. Microtubule-like structures were occasionally present. By contrast, the chloroplasts of palisade cells were larger and elliptical and had distinct grana stacks of 8–10 thylakoids.

### Enzyme activities

**Ru$P_2$ Carboxylase.** Ru$P_2$ carboxylase activity was 1167 ± 150 mmol $kg^{-1}$ $hr^{-1}$ (mean ± SEM, $n$ = 16, two experiments) in palisade cells and 884 ± 26 mmol $kg^{-1}$ $hr^{-1}$ ($n$ = 7, one experiment) in spongy cells (Fig. 1*A*). A trace of activity was observed in epidermal cells of the abaxial leaf surface [41 ± 9 mmol $kg^{-1}$ $hr^{-1}$ ($n$ = 15, three experiments)], but the assay with upper epidermal cells did not detect activity [11 ± 7 mmol $kg^{-1}$ $hr^{-1}$ ($n$ = 6, one experiment)]. The average of Ru$P_2$ carboxylase activity in all experiments with epidermal cells was 33 ± 7 mmol $kg^{-1}$ $hr^{-1}$ (Fig. 1*A*). Ru$P_2$ carboxylase activity was not detected in guard cells from the lower epidermis with either the less sensitive assay [15 ± 22 mmol $kg^{-1}$ $hr^{-1}$ ($n$ = 18, two experiments)] or the more sensitive version [7 ± 10 mmol $kg^{-1}$ $hr^{-1}$ ($n$ = 13, two experiments)]. Guard cells from the upper epidermis also lacked Ru$P_2$ carboxylase activity [−5 ± 3 mmol $kg^{-1}$ $hr^{-1}$ ($n$ = 15, two experiments)]. The average value for guard cells from both surfaces was 0 ± 5 mmol $kg^{-1}$ $hr^{-1}$ with the most sensitive version of the assay.

Ru$P_2$ carboxylase in extracts of guard cell protoplasts could not be detected [0.45 ± 3.5 μmol/mg of chlorophyll per hr (three experiments) data not shown]. By comparison, whole leaflet values were 406 ± 23 μmol/mg of chlorophyll per hr (three experiments).

In one experiment, guard cells were dissected from freeze-dried tobacco leaf (*Nicotiana glauca*) and assayed for Ru$P_2$ carboxylase activity. The value was insignificant [−30 ± 16 mmol $kg^{-1}$ $hr^{-1}$ ($n$ = 7, one experiment) data not shown].

***P*-Ribulokinase.** *P*-Ribulokinase activity was high in the photosynthetic parenchyma (Fig. 1*B*). In palisade cells, the activity was 17,230 ± 1382 mmol $kg^{-1}$ $hr^{-1}$ ($n$ = 16, two experiments), whereas it was 8772 ± 741 mmol $kg^{-1}$ $hr^{-1}$ ($n$ = 13, two experiments) in the spongy parenchyma. In comparison, the activity in guard cells and epidermal cells (lower leaf surface), was exceedingly low or insignificant [183 ± 77 mmol $kg^{-1}$ $hr^{-1}$ ($n$ = 10, one experiment) and −173 ± 296 mmol $kg^{-1}$ $hr^{-1}$ ($n$ = 13, one experiment), respectively].

**Glyceraldehyde-*P* Dehydrogenase ($NADP^+$) (Phosphorylating).** Glyceraldehyde-*P* dehydrogenase ($NADP^+$) showed the same relative distribution in the photosynthetic cells as the other two enzymes (Fig. 1*C*); it was higher in the palisade parenchyma [1218 ± 92 mmol $kg^{-1}$ $hr^{-1}$ ($n$ = 13, two experiments)] than in the spongy parenchyma [574 ± 158 mmol $kg^{-1}$ $hr^{-1}$ ($n$ = 6, one experiment)]. In epidermal cells from the lower leaf surface, the activity was insignificant [15 ± 16 mmol $kg^{-1}$ $hr^{-1}$ ($n$ = 6, one experiment)]. The activity in guard cells (abaxial surface) was 16 ± 11 mmol $kg^{-1}$ $hr^{-1}$ [$n$ = 11, two experiments (Fig. 1C)].

### Immunoelectrophoresis for Ru$P_2$ carboxylase

Clearly defined "rockets" were obtained with leaf extracts of *Festuca* and *V. faba* (lanes 1, 2, and 6–8, respectively, Fig. 2). Ru$P_2$ carboxylase protein was not detected in extracts of guard cell protoplasts (lanes 3–5, Fig. 2) at the same chlorophyll concentration.

## DISCUSSION

Earlier studies have been conflicting in regard to photosynthesis in guard cells. Pearson and Milthorpe (7) exposed leaves to $^{14}CO_2$ and subsequently assayed epidermal peels for $^{14}C$. They concluded that the rate of photosynthesis (chloroplast volume basis) was 6-fold higher in guard cells than in palisade cells of either *Vicia* or *Commelina*. In 1977 Thorpe and Milthorpe (26) exposed leaves to $^{14}CO_2$. They subsequently took epidermal peels ("attached epidermis") and assayed for $^{14}C$. They com-

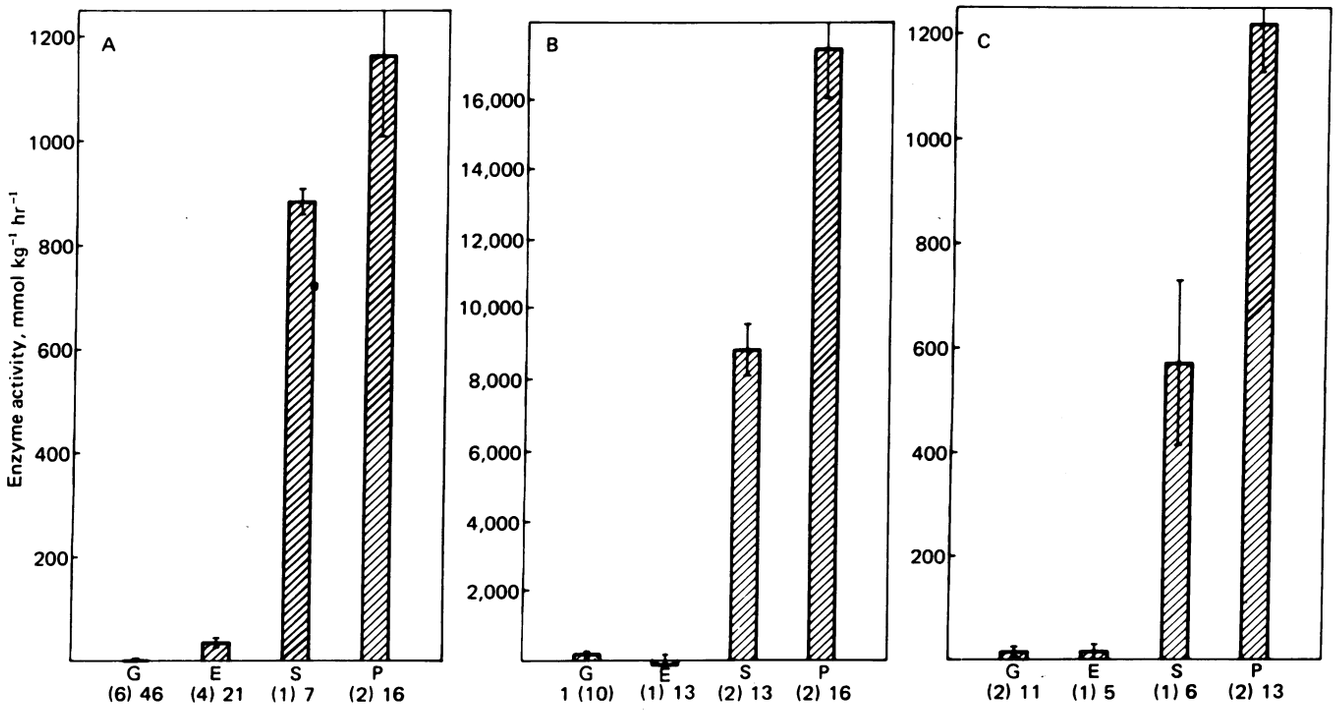


FIG. 1. Activities of Ru$P_2$ carboxylase (*A*), *P*-ribulokinase (*B*), and glyceraldehyde-*P* dehydrogenase ($NADP^+$) (phosphorylating) (*C*) in guard cells (G), epidermal cells (E), spongy parenchyma cells (S), and palisade parenchyma cells (P) dissected from freeze-dried *V. faba* leaflet. Error bar is SEM. The numbers below each bar indicate the number of experiments (in parentheses) and the total number of tissue samples analyzed.

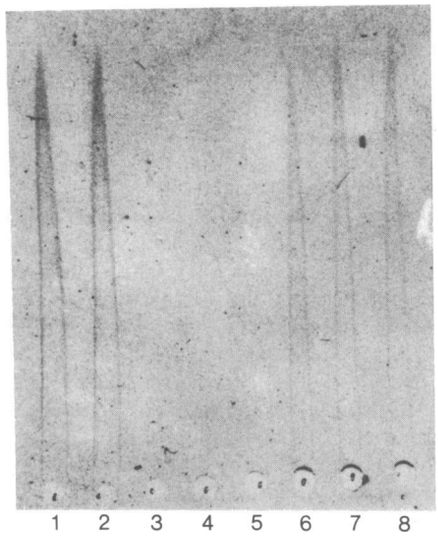


FIG. 2. Immunoelectrophoresis for Ru$P_2$ carboxylase in extracts of *Festuca* leaf (lanes 1 and 2), *V. faba* guard cell protoplasts (lanes 3–5), and *V. faba* leaflet (lanes 6–8). Extracts containing about 60 ng of chlorophyll were run in each lane.

pared the rate of $^{14}C$ accumulation in attached epidermis to that in epidermal peaks that were taken prior to exposure to $^{14}CO_2$ ("detached epidermis"). They attributed the greater accumulation of $^{14}C$ in attached epidermis than in detached epidermis to leakage from the latter. These results were recently reinterpreted (27) and attributed to transport, owing to the rapidity and magnitude of $^{14}C$ exchange between the mesophyll and epidermis (28, 29). Willmer and Dittrich (30) found radioactivity in intermediates of the photosynthetic carbon reduction pathway in extracts of $^{14}CO_2$-labeled epidermal peels of *Tulipa* and *Commelina*. Their experimental protocol was refined and the experiments were repeated by Raschke and Dittrich (31). The latter used great care to avoid mesophyll contamination of *Commelina* epidermal peels by microscopically examining each peel prior to exposure to $^{14}CO_2$. Their results indicated that the photosynthetic carbon reduction pathway was absent in these peels. However, they found that $^{14}CO_2$ was metabolized through intermediates of the photosynthetic carbon reduction pathway by epidermal peels of *Tulipa*, but they attributed this to contamination by mesophyll cells. The conflicting reports point to the difficulty of extrapolating $^{14}CO_2$ labeling data of epidermal peels to guard cells. The reason for this difficulty is clear. In *Vicia*, for example, ordinary epidermal cells have phospho*enol*pyruvate carboxylase and account for about 95% of the epidermal volume. Only guard cells have well-developed chloroplasts. Thus, even if guard cells did metabolize $^{14}CO_2$ via Ru$P_2$ carboxylase, the relative radioactivity incorporated by this mechanism in epidermal strips would be modest compared to that incorporated via phospho*enol*pyruvate carboxylase. Adding to the difficulty of interpreting the data is the presence of contaminating mesophyll cells that adhere to the strip during peeling.

Several groups have looked for the enzymes of the photosynthetic carbon reduction pathway in epidermal peels. One group (31) found low values for Ru$P_2$ carboxylase and *P*-ribulokinase activity in extracts of epidermal peels of *Commelina*. Unfortunately, their finding of low activity of Ru$P_2$ carboxylase is questionable because their extraction and assay procedure would not have resulted in the activated enzyme. In addition, the extremely high concentration of Ru$P_2$ (≈8 mM) used in the assay could contain significant amounts of inhibitory impurities (see ref. 32). Their assay for *P*-ribulokinase was also a measure of 3-*P*-glycerate formation. Because of their low endogenous levels of Ru$P_2$ carboxylase, the omission of an analytical coupling enzyme calls their negative results for this enzyme into question also. Willmer *et al.* (33) reported as much Ru$P_2$ carboxylase activity (chlorophyll basis) in epidermal peel extracts as whole leaf extracts of *Tulipa* and *Commelina*. Thorpe *et al.* (34) reported less Ru$P_2$ carboxylase activity in epidermal strips than in leaf extracts of *Commelina* but 2.5 times more Ru$P_2$ carboxylase protein (chlorophyll basis).

Our results show that the photosynthetic carbon reduction pathway must be absent in guard cell chloroplasts of *V. faba* (Figs. 1 and 2). A coordinated light and electron microscope study of the guard cells (data not shown) agreed with the previous reports on abundance of chloroplasts (7) and their fine structure (35). Additionally, the lack of Ru$P_2$ carboxylase in *Nicotiana* guard cells shows that the absence of this pathway is not unique to *Vicia* stomatal apparatus. We interpret previous reports of significant amounts of Ru$P_2$ carboxylase in epidermal peels to be due to contamination.

These results increase our understanding of guard cell metabolism. They show that guard cells, like ordinary epidermal cells, depend on the photosynthetic parenchyma for nutrition. Starch synthesis is enhanced in photosynthetic cells upon illumination due to stimulation of ADP-glucose pyrophosphorylase by increased levels of intermediates of the photosynthetic carbon reduction pathway (e.g., refs. 36 and 37). Unlike starch concentration in photosynthetic cells, that in guard cells decreases upon illumination (6). Thus, the absence of the photosynthetic carbon reduction pathway in guard cells may uncouple starch metabolism from illumination. However, starch metabolism is also regulated by inorganic phosphate (38), so the present results provide only a partial answer to this question.

These results raise an important question. Guard cell chloroplasts do not reduce $CO_2$; what, then, is their function? At present, it is not possible to give a complete answer because our knowledge of guard cell metabolism is so limited. The plastids obviously serve as a repository for starch, but this does not explain why they are pigmented. It is possible that chloroplasts supply reducing power to the cell, but this reducing power is not obligatory for functioning, because even stomatal apparatus which have chloroplasts can open in darkness. Furthermore, the presence of photosystem II (the $H_2O$-splitting act) has not been unequivocally demonstrated in guard cell chloroplasts; in fact, the scarcity of grana stacks in guard cell chloroplasts suggests a deficiency of photosystem II. Few data are available. Lurie (39) found higher chlorophyll a/b ratio in epidermal peels of *Vicia* then in whole leaf. This suggests enrichment of photosystem I. However, she found photosystem II activity to be comparable to that in whole leaf. The meaning of these data for guard cells is not clear, however. The epidermal strips "showed 2% contamination by mesophyll cells." Thus mesophyll chloroplasts were at least as abundant as guard cell chloroplasts in the epidermal peels. There must be basic biochemical functions of the chloroplasts (as opposed to simply amyloplasts), but these functions remain unspecified.

The biochemical similarity between guard cells and the other two chlorophyll-containing cell types known to lack Ru$P_2$ carboxylase is tenuous. Mesophyll cells of $C_4$ plants lack *P*-ribulokinase and Ru$P_2$ carboxylase but do contain the reductive step enzyme [glyceraldehyde-*P* dehydrogenase ($NADP^+$)] and both photosystems. The photosynthetic activity of these cells is coordinated with bundle sheath cells, which are deficient in photosystem II but have the full complement of enzymes of the photosynthetic carbon reduction pathway; the advantage is that $CO_2$ is enriched with respect to its competitive inhibitor, $O_2$, at the site of Ru$P_2$ carboxylase. The other chlorphyll-containing

cell type lacking Ru$P_2$ carboxylase is the heterocyst of Cyanobacteria. These cells are specialized for nitrogen fixation, which requires a strictly anaerobic atmosphere (see ref. 40 for review). Thus, they lack photosystem II and consequently, without photogeneration of reductant, do not contain the enzymes of the photosynthetic carbon reduction pathway.

The demonstration of high activity of the enzymes of the photosynthetic carbon reduction pathway in the palisade parenchyma adds further support to the belief that it is the major photosynthetic cell type (41, 42).

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