Mutants of the cruciferous plant *Arabidopsis thaliana* lacking glycine decarboxylase activity

C. R. SOMERVILLE* and W. L. OGREN†
*Department of Genetics, University of Alberta, Edmonton, Alberta, Canada T6G 2E9, and
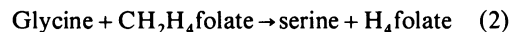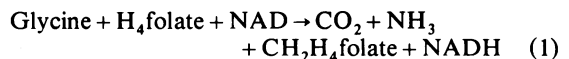†Department of Agronomy, University of Illinois, Urbana, IL 61801, and U.S. Department of Agriculture,
Agricultural Research Service, Urbana, IL 61801, U.S.A.*

A mutant of *Arabidopsis thaliana* (L.) Heyn. (a small plant in the crucifer family) that lacks glycine decarboxylase activity owing to a recessive nuclear mutation has been isolated on the basis of a growth requirement for high concentrations of atmospheric CO_2. Mitochondria isolated from leaves of the mutant did not exhibit glycine-dependent O_2 consumption, did not release $^{14}CO_2$ from [^{14}C]glycine, and did not catalyse the glycine–bicarbonate exchange reaction that is considered to be the first partial reaction associated with glycine cleavage. Photosynthesis in the mutant was decreased after illumination under atmospheric conditions that promote partitioning of carbon into intermediates of the photorespiratory pathway, but was not impaired under non-photorespiratory conditions. Thus glycine decarboxylase activity is not required for any essential function unrelated to photorespiration. The photosynthetic response of the mutant in photorespiratory conditions is probably caused by an increased rate of glyoxylate oxidation, which results from the sequestering of all readily transferable amino groups in a metabolically inactive glycine pool, and by a depletion of intermediates from the photosynthesis cycle. The rate of release of $^{14}CO_2$ from exogenously applied [^{14}C]glycollate was 14-fold lower in the mutant than in the wild type, suggesting that glycine decarboxylation is the only significant source of photorespiratory CO_2.

Mitochondria from the leaves of 'C₃' species catalyse a complex reaction in which two molecules of glycine are converted into one each of CO_2, NH_3 and serine, with a concomitant release of energy equivalent to one NADH molecule (Arron *et al.*, 1979; Bird *et al.*, 1972; Douce *et al.*, 1977; Kisaki & Tolbert, 1970; Kisaki *et al.*, 1971; Moore *et al.*, 1978; Woo & Osmond, 1976). Because the reaction has only been observed in preparations of intact plant mitochondria that are not amenable to direct biochemical analyses, details of the reaction mechanism are unknown. It has generally been assumed that the mechanism of the reaction is similar to, or identical with, glycine-cleavage reactions in bacteria and vertebrates. In these organisms, where it has been possible to purify the enzymes involved (Hiraga & Kikuchi, 1980*a,b*; Klein & Sagers, 1966, 1967; Kochi & Kikuchi, 1974; Motokawa & Kikuchi, 1974), the overall reaction has been subdivided into two sequential reactions described by the equations:

$$Glycine + H_4folate + NAD \rightarrow CO_2 + NH_3$$
$$+ CH_2H_4folate + NADH \quad (1)$$

$$Glycine + CH_2H_4folate \rightarrow serine + H_4folate \quad (2)$$

The reaction in eqn. (2) is catalysed by the enzyme serine hydroxymethyltransferase (EC 2.1.2.1), which is present in leaves as two isoenzymes (Shah & Cossins, 1970; Somerville & Ogren, 1981*a*; Woo, 1979), one of which is located in the mitochondrial matrix (Woo, 1979). The enzyme complex that catalyses eqn. (1), designated glycine synthase (EC 2.1.2.10), consists of four protein components that can be individually purified and reconstituted to

Abbreviations used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; CH_2H_4folate, N^5N^{10}-methylenetetrahydrofolic acid; H_4folate, tetrahydrofolic acid; Mops, 4-morpholinepropanesulphonic acid; Mes, 4-morpholine-ethanesulphonic acid; BSA, bovine serum album. The gene symbols *stm* and *glyD* represent genetic loci required for mitochondrial serine transhydroxymethylase and glycine decarboxylase activity respectively.

form a catalytically active complex (Hiraga & Kikuchi, 1980a,b; Klein & Sagers, 1966, 1967; Kochi & Kikuchi, 1974; Motokawa & Kikuchi, 1974). One of these four proteins, glycine decarboxylase, contains pyridoxal phosphate and catalyses the cleavage of glycine to CO_2 and methylamine without the addition of other cofactors (Hiraga & Kikuchi, 1980b). This protein also catalyses the exchange of bicarbonate with the carboxy group of glycine. Both activities are greatly stimulated by the addition of a lipoic acid-containing protein, aminomethyl carrier protein (Hiraga & Kikuchi, 1980a), which acts as an electron acceptor and as a carrier of the methylamine resulting from glycine decarboxylation. The presence of the other two components of the complex is required for the transfer of electrons to NAD, the release of NH_3 and the transfer of the methylene group to H_4folate (Klein & Sagers, 1966, 1967; Kochi & Kikuchi, 1974; Motokawa & Kikuchi, 1974).

The functional significance of the glycine decarboxylation reaction in C_3 species is that most or all photorespiratory CO_2 is the direct product of this reaction (Canvin et al., 1976; Kisaki & Tolbert, 1970; Somerville & Ogren, 1981a; Woo & Osmond, 1976). To better understand the details of this reaction we have undertaken the characterization of mutants of Arabidopsis thaliana (a small plant in the crucifer family) in which photorespiratory glycine accumulates as a metabolically inactive end product of photosynthesis. We have previously described one class of such mutants lacking mitochondrial serine hydroxymethyltransferase activity (Somerville & Ogren, 1981a). These mutants were unable to decarboxylate glycine because they could not regenerate the H_4folate required as a C_1-acceptor in the decarboxylation reaction. In the present paper we describe a new class of mutants, designated glyD, which appear to be defective in an earlier step of the decarboxylation reaction.

Materials and methods

Reagents

[1-^{14}C]Glycine and [1-^{14}C]glycollic acid were obtained from Amersham Corp., Arlington Heights, IL, U.S.A. $NaH^{14}CO_3$ was obtained from New England Nuclear Corp., Boston, MA, U.S.A. Cellulysin and Macerase were obtained from Calbiochem–Behring Corp., San Diego, CA, U.S.A. All other reagents were of the highest purity available from Sigma, St. Louis, MO, U.S.A.

Plant material and growth conditions

All lines of Arabidopsis thaliana (L.) Heyn. described here are descended from the Columbia wild type (Rédei, 1970). The mutant line CS64 (stm) has been previously characterized as lacking mitochondrial serine hydroxymethyltransferase activity

(Somerville & Ogren, 1981a). The mutant line CS116 (glyD) was isolated as described previously (Somerville & Ogren, 1979, 1981b) on the basis of a growth requirement for high CO_2. Experiments were performed on material that had been advanced at least five generations after the original selection. Plants were grown under continuous fluorescent illumination ($200 \mu E \cdot m^{-2} \cdot s^{-1}$) at 22°C and 75% relative humidity on a Perlite/Vermiculite/sphagnum (1:1:1) mixture irrigated with mineral nutrients (Somerville & Ogren, 1981b).

Gas-exchange measurements

Photosynthesis was measured by i.r. gas analysis with intact plants in an open system (Somerville & Ogren, 1979, 1981a,b).

Labelling studies

Plants were labelled with $^{14}CO_2$ and products were analysed by combined ion-exchange and paper or thin-layer chromatography as previously described (Somerville & Ogren, 1979, 1981a,b).

Isolation of mitochondria

Coupled mitochondria were isolated from protoplasts by minor modifications of methods previously described for the isolation of chloroplasts from Arabidopsis (Somerville et al., 1981). Approx. 4 g of leaf material was finely divided by chopping with a razor in 100 ml of 0.5 M-sorbitol/20 mM-Mes (adjusted to pH 5.5 with KOH)/1 mM-$CaCl_2$/0.1% bovine serum albumin/2% (w/v) Cellulysin/1.3% macerase. After 3–4 h of digestion at room temperature, the mixture was filtered through a 200 μm net and the protoplasts collected by centrifugation at 200 g for 5 min. The pellet was resuspended in 0.42 M-sorbitol/60% (v/v) Percoll/10 mM-Mes (pH 5.5)/1 mM-$CaCl_2$/0.1% bovine serum albumin overlaid with 0.5 M-sorbitol/10 mM-Mes (pH 5.5)/0.1% BSA/1 mM-$CaCl_2$ and centrifuged at 200 g for 5 min in a swinging-bucket rotor. The band of intact protoplasts at the Percoll/sorbitol interface was removed, diluted with upper buffer, and re-centrifuged. The protoplasts were resuspended at approx. 0.1 mg of chlorophyll·ml^{-1} in 0.38 M-sorbitol/20 mM-Hepes (pH 7.5)/10 mM-EDTA/0.1% BSA and ruptured by passage through a 15 μm nylon net. Chloroplasts were removed by centrifugation at 1000 g for 3 min and mitochondria pelleted by centrifugation at 8000 g for 20 min. The mitochondrial pellet, which lacked measurable chlorophyll, was resuspended in 0.38 M-sorbitol/20 mM-Mops (pH 7.2)/0.1% BSA by gentle agitation for a period of about 30 min at 4°C. The suspension was then centrifuged for 15 s in a Microfuge to yield a completely colourless preparation, which was kept on ice until use. The procedure yielded about 300 μg of mitochondrial protein per mg of protoplast

chlorophyll. All buffers for isolation and assay were filter-sterilized.

Mitochondrial assays

Substrate-dependent O_2 consumption was measured in a Clark-type oxygen electrode (Hansatech) by adding $200\,\mu l$ of mitochondrial suspension (about $100\,\mu g$ of protein) to $600\,\mu l$ of assay buffer containing $0.36\,\text{M-sorbitol}/20\,\text{mM-Mops}$ (pH 7.2)/ $8\,\text{mM-KCl}/4\,\text{mM-NaH}_2\text{PO}_4$ (pH 7.2)/$4\,\text{mM-MgCl}_2$/ 0.1% BSA. State-2 rates of O_2 consumption were initiated by the addition of glycine or sodium malate, pH 7.2, to a final concentration of 8 mM. State-3 rates were measured after the addition of $4\,\mu l$ of 30 mM-ADP. P/O and respiratory-control ratios were determined by standard methods.

Glycine–bicarbonate exchange was measured as glycine-dependent $^{14}CO_2$ fixation (Clandinin & Cossins, 1975; Hiraga & Kikuchi, 1980*b*; Klein & Sagers, 1966). The $300\,\mu l$ reaction mixtures contained $20–50\,\mu g$ of mitochondrial protein in assay buffer, which contained, in addition, 10 mM-glycine and $33\,\text{mM-NaH}^{14}\text{CO}_3$ ($2\,\mu\text{Ci/mol}$). After a period of incubation at $22\,^\circ\text{C}$, reactions were terminated with $200\,\mu l$ of 6 M-acetic acid, dried at $60\,^\circ\text{C}$, resuspended in water and radioactivity was determined by liquid-scintillation counting. Control reaction mixtures lacked glycine. The mitochondria used in these experiments had respiratory-control ratios of more than 1.9 with malate as the substrate. The radioactive products of the fixation reaction were determined to be glycine and serine by chromatographic analysis of parallel reactions.

Glycine decarboxylation was measured by adding $8\,\text{mM-}[1\text{-}^{14}\text{C}]$glycine ($3.5\,\mu\text{Ci}/\mu\text{mol}$) to $300\,\mu l$ of assay buffer containing $30–50\,\mu g$ of mitochondrial protein. Reaction mixtures were placed in small cups suspended over $700\,\mu l$ of 10% (v/v) triethanolamine in serum-stoppered 20 ml scintillation vials. Reactions were initiated with glycine and terminated by injecting $100\,\mu l$ of 6 M-acetic acid into the reaction mixture. The vials were left overnight to permit trapping of $^{14}CO_2$, the reaction cups removed, and the trapped ^{14}C radioactivity determined by scintillation counting.

Mitochondrial protein was determined with a dye-binding assay (Spector, 1978).

Swelling experiments

Mitochondrial transport was examined by the osmotic-swelling technique described by (Cavalieri & Huang, 1980). Before use, the mitochondria were preincubated in $0.38\,\text{M-sorbitol}/20\,\text{mM-Mops}$ (pH 7.2)/0.1% BSA/rotenone ($1.4\,\mu g\cdot\text{ml}^{-1}$)/antimycin A ($1\,\mu g\cdot\text{ml}^{-1}$) at $4\,^\circ\text{C}$. Swelling was measured by placing $250\,\mu l$ of mitochondria ($150\,\mu g$ of protein) in a cuvette with a 1 cm light path, then adding $250\,\mu l$ of 400 mM-glycine or -sucrose and measuring

the change in A_{520}. The effect of mersalyl was examined by preincubating the mitochondrial suspension in medium containing mersalyl at a final concentration of 0.2 mM for 10 min at $4\,^\circ\text{C}$.

$^{14}CO_2$ release from glycollate

The lower epidermis was removed from leaves, which were then placed on a 0.35% agarose gel containing 20 mM-Mes, pH 5.5, 0.25 M-sorbitol and $10\,\mu\text{Ci}$ of $[1\text{-}^{14}\text{C}]$glycollate ($8.3\,\mu\text{Ci}\cdot\mu\text{mol}^{-1}$). The gel was placed in a Plexiglass chamber, similar to that described by Atkins & Canvin (1971), which was illuminated ($400\,\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) from above. The chamber was maintained at $24\,^\circ\text{C}$ by submersion in a water bath and shielded from i.r. radiation by an optical filter. The chamber was gassed at $28\,\text{ml}\cdot\text{min}^{-1}$ with O_2/N_2 (1:1) at 75% relative humidity so that the chamber volume was exchanged approx. ten times/min. The gas exited from the chamber to a hypodermic needle which was inserted through a serum stopper into 10 ml of a CO_2-trapping mixture (270 ml of methanol, 270 ml of phenethylamine, 460 ml of toluene, 6 g of 2,5-diphenyloxazole) contained in a 20 ml scintillation vial. The exhaust port of this vial was similarly connected in series to a second vial containing the trapping mixture. At timed intervals, the needle was rapidly transferred to a new set of trapping vials. At the end of the experiment, the $^{14}CO_2$ trapped was determined by direct liquid-scintillation counting of the vials. Control experiments indicated that, under these conditions, more than 96% of the CO_2 was trapped in the first vial so that the combination of two traps in series was essentially quantitative.

Results

Labelling studies

Among the mutants of *Arabidopsis* originally recovered on the basis of a growth requirement for high concentrations of atmospheric CO_2 (Somerville & Ogren, 1979), several were found to accumulate high concentrations of $[^{14}\text{C}]$glycine during $^{14}CO_2$ fixation under standard atmospheric conditions. We have previously described one class of such mutants (designated *stm*) as lacking mitochondrial serine hydroxymethyltransferase activity (Somerville & Ogren, 1981*a*). A second class of glycine-accumulating mutants, designated *glyD*, was initially distinguished from the *stm* mutants by the presence of wild-type activities of serine hydroxymethyltransferase activity (results not shown). The inability of the *glyD* mutants to metabolize glycine in a normal fashion is apparent from the results presented in Fig. 1. In this experiment, wild-type *Arabidopsis* and the *glyD* mutant CS116 were illuminated for 10 min in an atmosphere containing $^{14}CO_2$ (350–$400\,\mu l\cdot\text{litre}^{-1}$) and 21% O_2, then the atmosphere
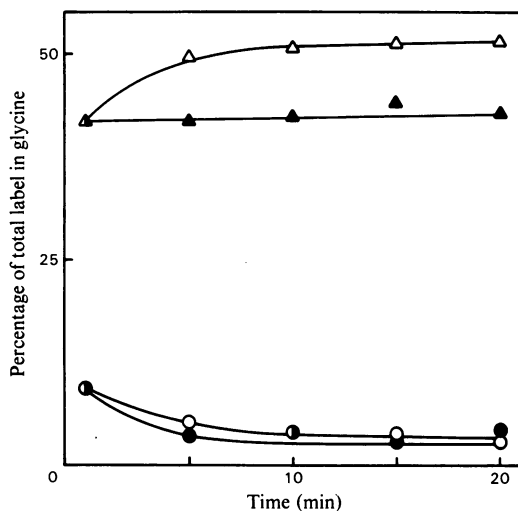
Fig. 1. *Persistence of labelled glycine in wild-type* (O, ●) *or glyD-mutant* (△, ▲) *Arabidopsis leaves during a light* (O, △) *or dark* (●, ▲) *chase with* $^{12}CO_2$
Intact plants were allowed to photosynthesize for 10 min in $^{14}CO_2/O_2/N_2$ (350–400 $\mu l \cdot$ litre^{-1}:21%: balance), then $^{12}CO_2$ in the same gas mixture was introduced and, at the indicated intervals, plants were removed to liquid N_2, extracted and the products identified and quantified. In one series of experiments the light was extinguished at zero time; in a parallel experiment, illumination was continued during the 20 min chase. Each point represents the average result for two independently treated plants.

was rapidly changed to air containing $^{12}CO_2$. Plants were removed for product analysis after various times (0–20 min) of exposure to light or dark conditions. In the wild type, the proportion of label in glycine rapidly declined from about 9% of the total at the end of the labelling period (time zero) to about 3.5% after a 20 min light or dark chase. In contrast, the proportion of label represented by glycine in the mutant remained constant at about 42% during a dark chase, and increased slightly (to about 50%) during a light chase, owing to continued flow of labelled carbon from the Calvin cycle. These results clearly indicated that the *glyD* mutant was unable to metabolize glycine at normal rates. In this respect the *glyD* and *stm* mutants were indistinguishable (see Somerville & Ogren, 1981*a*).

Genetic analysis

The frequency of the mutant phenotype in segregating populations was determined by scoring for chlorosis and cessation of growth after a change in atmospheric conditions (Somerville & Ogren, 1981*b*). Of 465 F_2 progeny from a wild type × CS116 (*glyD*) cross, 110 showed the mutant phenotype,

and 355 were of wild-type phenotype. The 3:1 segregation (χ^2 0.47, $P > 0.5$) indicates that a single recessive nuclear mutation is responsible for the phenotype. The F_1 progeny of reciprocal crosses between the wild type and CS116 (*glyD*) showed wild-type phenotypes. Similarly, the F_1 progeny of crosses between lines CS64 (*stm*) and CS116 (*glyD*) were of wild-type phenotype. The existence of genetic complementation in crosses between these lines is considered convincing evidence that *stm* and *glyD* represent distinct loci.

Isolation of mitochondria from protoplasts

The small size of *Arabidopsis* limits the utility of methods of organelle isolation that require large (>10 g) amounts of leaf material. For this reason mitochondria were isolated by rupturing purified leaf protoplasts. Procedures commonly used in the isolation of mitochondria from other species (Arron et al., 1979; Douce et al., 1977; Moore et al., 1978) resulted in uncoupled mitochondria of very low activity (results not shown). However, the addition of 10 mM-EDTA to the protoplast-disruption medium resulted in the recovery of coupled mitochondria that exhibited high rates of malate oxidation (Table 1). EDTA was also required for the isolation of active chloroplasts from *Arabidopsis* (Somerville et al., 1981) and several other species. Although the reason for this requirement is unknown, it may be related to the presence of Ca^{2+}-activated enzymes that are released on cell rupture and rapidly destroy the functional integrity of organelle membranes (Tomomatsu & Asahi, 1980).

In contrast with mechanically isolated mitochondria, which are generally heavily contaminated with chlorophyll, the mitochondria isolated from protoplasts lacked measurable chlorophyll contamination. The method may, therefore, be of some advantage in studies requiring organelles of high purity.

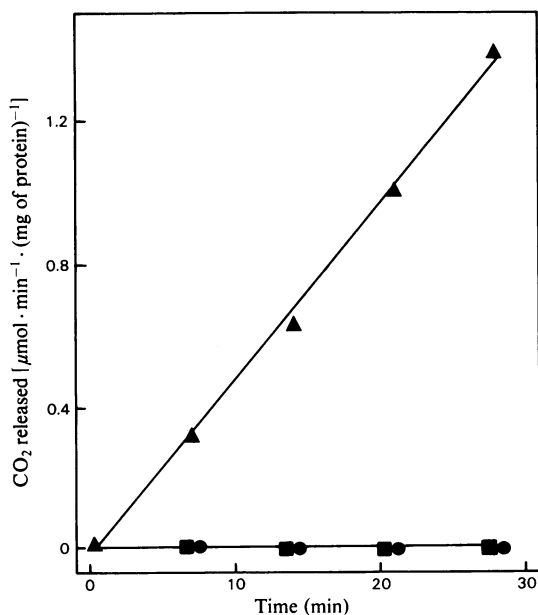### Glycine cleavage by isolated mitochondria

Mitochondria isolated from the wild type exhibited coupled glycine-dependent O_2 consumption at about 55% of the rate observed for malate at the same concentration (Table 1). In contrast, mitochondria from the *glyD* mutant CS116 or the *stm* mutant CS64 did not exhibit glycine-dependent O_2 consumption, although malate was oxidized at rates comparable with that shown by the wild type.

Comparison of the ability of mitochondria from the three lines to decarboxylate glycine produced similar results (Fig. 2). Mitochondria from the wild type exhibited rates of CO_2 release from glycine that were in excess of the State-2 rate of glycine-dependent O_2 consumption. Mitochondria from the *glyD* and *stm* mutants lacked measurable glycine decarboxylase activity. This result was consistent

Table 1. *Rates of malate- or glycine-dependent O_2 consumption of mitochondria from wild-type and mutant lines of Arabidopsis*

The values are averages for two independent assays. Abbreviation used: N/A, not applicable.

		O_2 consumption [nmol·min^{-1}·(mg of protein)$^{-1}$]		
Substrate	Line ...	Wild-type	CS116(*glyD*)	CS64(*stm*)
Malate				
State 2		63.0	87.6	94.1
State 3		107.3	143.5	164.0
State 4		55.9	85.8	90.7
P/O ratio ...		2.9	2.6	2.3
Glycine				
State 2		37.7	0	0
State 3		58.9	0	0
State 4		36.0	N/A	N/A
P/O ratio ...		1.8	N/A	N/A

Fig. 2. *Rate of glycine decarboxylation by isolated mitochondria from wild type* (▲)*, glyD mutant CS116* (■) *and stm mutant CS64* (●)
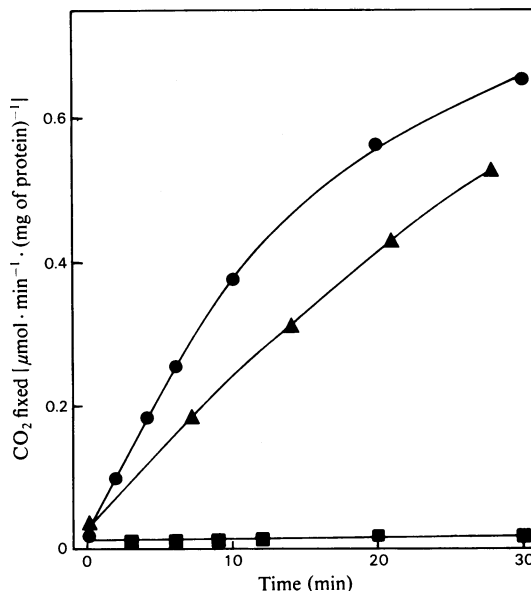
Fig. 3. *Rate of glycine–bicarbonate exchange catalysed by mitochondria from wild type* (▲)*, glyD mutant CS116* (■) *and stm mutant CS64* (●)

with the apparent metabolic inactivity of photorespiratory glycine in these mutant lines.

Mitochondria from leaves of C_3 species have previously been shown to catalyse the exchange of bicarbonate with the carboxy group of glycine (Clandinin & Cossins, 1975; Woo & Osmond, 1976). By analogy with the model deduced from other organisms (Hiraga & Kikuchi, 1980*a,b*; Klein & Sagers, 1966; Kochi & Kikuchi, 1974; Motokawa & Kuchi, 1974), this activity is a measure of the first step of the overall glycine decarboxylase reaction and is independent of the availability of the cofactors NAD and H_4folate. Mitochondria from wild-type *Arabidopsis* and the *stm* catalysed glycine–bicarbonate exchange at comparable rates, whereas mitochondria from the *glyD* mutant line CS116 did not exhibit this activity (Fig. 3). These results, which clearly distinguish between the *stm* and *glyD* mutants, suggest that the *glyD* mutant is deficient in one of the two proteins required for this activity. The ability of the *stm* mutant to catalyse the exchange reaction but not the decarboxylation reaction is consistent with the proposal (Somerville & Ogren, 1981*a*) that glycine cleavage is blocked in this mutant at the level of H_4folate regeneration.

Mitochondrial transport of glycine

The inability of the *glyD* mutant to catalyse the glycine–bicarbonate exchange could, in principle, be due to an inability to transport glycine into mitochondria. This possibility was investigated by employing the osmotic-swelling technique, which is widely used to study transport phenomena in mitochondria. The results of this experiment (Fig. 4) indicate that glycine rapidly entered mitochondria from the *glyD* mutant CS116 but, by the same criteria, sucrose did not. Thus the inability of mitochondria from this line to catalyse the glycine–bicarbonate exchange does not appear to be due to a permeability barrier.

Arguments have been presented for (Cavalieri & Huang, 1980; Dench *et al.*, 1978) and against (Day & Wiskich, 1980) the existence of a carrier-mediated transport mechanism for glycine transport in plant mitochondria. This basis of the discrepancy is the apparently variable effect of mersalyl on glycine-induced swelling. The results presented in Fig. 4 indicate that preincubation of mitochondria in 0.2 mM-mersalyl inhibited glycine-induced swelling. In this respect our results support the proposal (Cavalieri & Huang, 1980) that glycine transport is carrier-mediated in plant mitochondria. It may be worth noting that Day & Wiskich (1980) did not preincubate their mitochondrial preparations with mersalyl before examining the effect on glycine uptake.

Physiological effects

The *glyD* mutant line CS116 was phenotypically indistinguishable from the wild type when maintained under atmospheric conditions that prevent the initial reaction in photorespiration, oxygenation of ribulose 1,5-bisphosphate (results not shown). These conditions are high CO_2 concentration (e.g. $CO_2/O_2/N_2$, 1:21:78) or low O_2 concentrations,
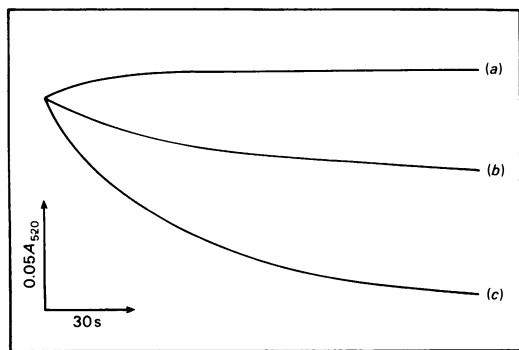
such as $CO_2/O_2/N_2$, 350 μl/litre, 2% and balance respectively. The effect of the *glyD* mutation was, however, readily apparent when photosynthesis was measured under photorespiratory conditions (Fig. 5). Under these conditions the photosynthesis rate began to decline after 3–4 min of illumination and continued to decline until a plateau was reached at about 30% of the wild-type rate. If, at this point, the plant was placed in darkness for a short period (15 min), the subsequent rate of photosynthesis was greater than the plateau rate. This dark-induced recovery, which was similar to that observed under similar circumstances in other mutants with defects in photorespiratory carbon metabolism (Somerville & Ogren, 1979, 1981*a*), is considered evidence that it is not glycine accumulation in itself that inhibits photosynthesis, since the glycine pool in the mutant did not change appreciably during 20 min of darkness (Fig. 1).

Glycollate metabolism

Studies of glycollate metabolism in the presence of inhibitors have indicated the possibility that there are perhaps two sources of photorespiratory CO_2, namely glycine oxidation and glyoxylate oxidation (Oliver, 1979; Servaites & Ogren, 1977). In contrast, gas-exchange studies on *Arabidopsis stm* mutants indicated that glycine oxidation was the only significant source of photorespiratory CO_2 (Somerville & Ogren, 1981*a*). The *glyD* mutant, which completely lacks glycine decarboxylase activity, provided an additional method to examine the metabolic fate of glycollate. A low concentration of high-specific-activity [1-^{14}C]glycollate was supplied to detached leaves of wild-type and *glyD Arabidopsis*. The wild
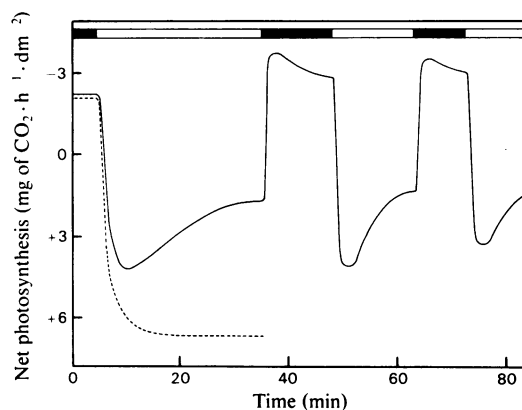
Fig. 4. *Mersalyl inhibition of mitochondrial swelling induced by glycine*
(*a*) Sucrose; (*b*) glycine and mersalyl; (*c*) glycine.

Fig. 5. *Net photosynthetic CO_2 fixation by wild-type and glyD-mutant-CS116 Arabidopsis*
The gas stream contained $CO_2/O_2/N_2$ (398 μl·litre^{-1}:50%:balance). Conditions of illumination: ▭, light; ▬, dark. ——, Response of the mutant line CS116; – – – –, wild-type response.
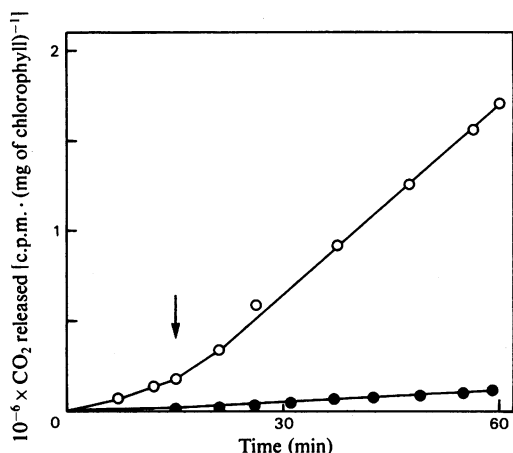
Fig. 6. $^{14}CO_2$ *release from* $[1-^{14}C]$*glycollate by detached leaves of wild-type* (O) *and glyD-mutant-CS116* (●) *Arabidopsis*
The leaves were illuminated at the time indicated by the arrow.

type released $^{14}CO_2$ at a rate 14-fold greater than that observed in the mutant (Fig. 6). This result indicates that leaf cells possess no major mechanism for the photorespiratory catabolism of glycollate to CO_2 other than by glycine decarboxylation.

Discussion

By all criteria examined, the *glyD* mutant appears to be defective in one of the components involved in catalysing the first step of the glycine decarboxylase reaction. The only apparent effect of this lesion was an inability of the mutant to metabolize photorespiratory glycine. In this respect, the *glyD* mutant is functionally equivalent to the previously described *stm* mutants (Somerville & Ogren, 1981a). In both mutant classes, photosynthesis is unimpaired under non-photorespiratory conditions, but reversibly inhibited in atmospheres that permit the diversion of carbon into the photorespiratory pathway. Consideration of the results presented in Figs. 1 and 5 suggests that it is not glycine accumulation in itself that inhibits photosynthesis, since recovery of photosynthetic CO_2 fixation occurred after a 15 min dark treatment, whereas no diminution of the glycine pool is evident during this time. We previously suggested, in the case of *stm* mutants, that inhibition of photosynthesis could be due, in part, to the accumulation of all readily transferable NH_3 groups in the form of metabolically inactive glycine (Somerville & Ogren, 1981a). In the absence of amino donors for the transamination of glyoxylate, glyoxylate is oxidized to CO_2. The net effect

of glyoxylate oxidation is an increase in the rate of photorespiration and a corresponding decrease in apparent photosynthesis. The importance of amino donors, in this respect, has recently been substantiated by the results of experiments with isolated soya-bean (*Glycine max*) cells in which glyoxylate decarboxylation was essentially eliminated by the provision of exogenous amino donors for glyoxylate amination (Oliver, 1981). Photosynthesis may also be decreased in the mutant because of a drain of carbon from the photosynthetic cycle due to the non-recycling of glycollate carbon.

In addition to the *glyD* mutant discussed here, we have isolated two other mutants with identical characteristics that fail to complement the mutant line CS116 in genetic crosses. Thus the additional lines carry allelic mutations of the *glyD* locus. Since at least four proteins appear to be involved in the decarboxylation reaction in other organisms, it may be possible to identify at least three other classes of complementing mutants. As we have not yet identified such mutants among the relatively large (>40) number of photorespiratory mutants already characterized, it remains possible that, unlike the *glyD* gene product, other components of the glycine decarboxylase complex are required for some function unrelated to photorespiration. Alternatively, it is also possible that the glycine synthase activity in plants involves a substantially different mechanism than in bacteria or vertebrates. Some degree of difference may be indicated by the relative lability of the activity in plants as compared with other systems (Clandinin & Cossins, 1975; Kisaki *et al.*, 1971; Woo & Osmond, 1976).

The results obtained by feeding $[1-^{14}C]$glycollate to wild-type and *glyD* leaves indicated that glycine decarboxylation is the only significant source of photorespiratory CO_2. This conclusion agrees with that reached from an examination of photorespiratory CO_2 evolution in an *Arabidopsis* mutant deficient in serine hydroxymethyltransferase activity (Somerville & Ogren, 1981), but contradicts conclusions reached from the study of photorespiratory carbon metabolism in the presence of a glycine decarboxylation inhibitor (Oliver, 1979; Servaites & Ogren, 1977). In the presence of the inhibitor, glycine is an end product of photorespiration and thus depletes the pool of readily transferable amino groups. Studies with the *stm* mutant demonstrated that when amino nitrogen accumulated in glycine, glyoxylate could not be aminated to glycine, so it was rapidly oxidized to CO_2 (Somerville & Ogren, 1981a). This interdependence of glycine decarboxylation and glyoxylate amination was not considered in the interpretation of the inhibitor experiments. The confounding difficulty of glyoxylate oxidation occurring as an artefact in the absence of glycine decarboxylation can be overcome by providing NH_3,

serine or glutamate exogenously (Oliver, 1981; Somerville & Ogren, 1981a). In the experiment reported here, depletion of the amino nitrogen pool was prevented by supplying a low concentration of glycollate to the leaf tissue.

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