Plant Physiol. (1981) 67, 666–671
0032-0889/81/67/0666/06/$00.50/0

Photorespiration-deficient Mutants of *Arabidopsis thaliana* Lacking Mitochondrial Serine Transhydroxymethylase Activity[1]

Received for publication July 30, 1980 and in revised form October 7, 1980

C. R. SOMERVILLE[2] AND WILLIAM L. OGREN[2, 3]
[2]*Department of Agronomy, University of Illinois, Urbana, Illinois 61801 and* [3]*United States Department of Agriculture, Science and Education Administration-Agricultural Research, Urbana, Illinois 61801*

ABSTRACT

Three allelic mutants of *Arabidopsis thaliana* which lack mitochondrial serine transhydroxymethylase activity due to a recessive nuclear mutation have been characterized. The mutants were shown to be deficient both in glycine decarboxylation and in the conversion of glycine to serine. Glycine accumulated as an end product of photosynthesis in the mutants, largely at the expense of serine, starch, and sucrose formation. The mutants photorespired CO_2 at low rates in the light, but this evolution of photorespiratory CO_2 was abolished by provision of exogenous NH_3. Exogenous NH_3 was required by the mutants for continued synthesis of glycine under photorespiratory conditions. These and related results with wild-type *Arabidopsis* suggested that glycine decarboxylation is the sole site of photorespiratory CO_2 release in wild-type plants but that depletion of the amino donors required for glyoxylate amination may lead to CO_2 release from direct decarboxylation of glyoxylate. Photosynthetic CO_2 fixation was inhibited in the mutants under atmospheric conditions which promote photorespiration but could be partially restored by exogenous NH_3. The magnitude of the NH_3 stimulation of photosynthesis indicated that the increase was due to the suppression of glyoxylate decarboxylation. The normal growth of the mutants under nonphotorespiratory atmospheric conditions indicates that mitochondrial serine transhydroxymethylase is not required in C_3 plants for any function unrelated to photorespiration.

Photorespiratory CO_2 is the product of a complex mitochondrial reaction in which glycine is converted to stoichiometric amounts of CO_2, NH_3, and the C_1 group of N^5,N^{10}-methylenetetrahydrofolate (10–12). In those organisms where it has been possible to solubilize the enzymes involved in the decarboxylation reaction, an absolute requirement for THF[4] has been demonstrated (13, 19). Regeneration of the THF from C_1-THF is accomplished in plant mitochondria by the enzyme serine transhydroxymethylase (EC 2.1.2.1), which catalyzes the following reversible reaction:

$$\text{glycine} + C_1\text{-THF} \rightleftharpoons \text{serine} + \text{THF}$$

Thus, in the over-all glycine decarboxylation reaction, 2 molecules glycine are converted to 1 molecule each of CO_2, NH_3, and serine.

Evidence suggesting that these reactions can account for most or all of the CO_2 released during photorespiration has been obtained by demonstrating the reaction in isolated mitochondria (3, 12, 18, 31), by comparing the specific activity of photorespiratory CO_2 to that of photorespiratory cycle intermediates (16), and by demonstrating, in the presence of chemical inhibitors, that the glycine to serine conversion is tightly coupled to photorespiratory CO_2 evolution (11, 14, 15). These experiments cannot unequivocally exclude the possibility that there are other sites of photorespiratory CO_2 release (4). Inhibitor studies are also plagued by nonspecific effects which preclude an appraisal of the long-term physiological effects of blocking the decarboxylation reaction.

It has been suggested (4, 7, 8, 15, 34) that, under certain circumstances, glyoxylate, the immediate precursor of photorespiratory glycine, may undergo decarboxylation to produce CO_2 and formate. This reaction has been demonstrated *in vitro* with isolated peroxisomes (7), but evidence substantiating the occurrence of this reaction *in vivo* is totally lacking. Here, we provide evidence that, under normal physiological conditions, glycine decarboxylation is the sole site of photorespiratory CO_2 release but that, under conditions of severe amino depletion, photorespiratory CO_2 may arise from direct decarboxylation of glyoxylate. This conclusion is based upon an analysis of mutants of *Arabidopsis* that lack mitochondrial serine transhydroxymethylase activity and are, therefore, defective in both glycine decarboxylation and the glycine to serine conversion.

MATERIALS AND METHODS

Plant Material and Growth Conditions. All lines of *Arabidopsis thaliana* (L.) Heynh. described here are descended from the Columbia wild-type (22). The procedure for isolation of the mutant lines (CS42, CS64, CS115) has been described (24). Plants were grown under continuous fluorescent illumination (200 μE m^{-2} s^{-1}) at 22 C and 75% RH on a perlite:vermiculite:sphagnum (1:1:1) mixture irrigated with mineral nutrient solution (26, 27). The atmosphere in the growth chamber was maintained at approximately 1% CO_2 in air by continual flushing with a gas mixture of this composition.

Gas-exchange Measurements. Methods for gas-exchange measurements on intact plants have been described (24, 27). Measurements on leaf fragments were performed with an open system similar to that described by Atkins and Canvin (2). The lower epidermis of the leaves was removed to facilitate uniform uptake of exogenous compounds. The stripped leaves then were placed on a 2-mm thick agarose gel which was supported on a membrane filter and contained 0.3 M sorbitol, 15 mM Mops (pH 7.5), 0.7% agarose, and other compounds as indicated in the text. Wild-type leaves treated in this-manner had about 80% of the photosynthesis rate observed with intact plants and showed no deterioration of photosynthetic capacity for 3.5 h. Temperature was maintained at 25 C and RH of the entering gas stream was 70%. Flow rate was monitored with a mass flowmeter and CO_2 exchange was monitored with an IR gas analyzer.

Labeling Studies. Product analyses and methods for labeling

[1] This work was supported in part by Grant 5901-0410-9-0341-0 from the United States Department of Agriculture Competitive Research Grants Office.

[4] Abbreviations: THF, tetrahydrofolic acid; C_1-THF, N^5,N^{10}-methylenetetrahydrofolic acid; RuBP, ribulose 1,5-bisphosphate.

intact plants with $^{14}CO_2$ have been described (24, 27). Plants were held in darkness for 1 to 2 h before use to permit equilibration of metabolite pools. Labeling of stripped leaf fragments was performed using the gas-exchange system described above. At the beginning of the labeling period, the system was closed and $^{14}CO_2$ was generated by injecting $NaH^{14}CO_3$ into an in-line reservoir of 8.5% phosphoric acid. Gas flow through the system was maintained with a diaphragm pump at a flow rate equivalent to 25 volume exchanges/min. The volume of the system was calibrated so that the CO_2 concentration declined by less than 10% during the course of the labeling period. At the end of the labeling experiment, the gels supporting the leaf fragments were rapidly (3 to 5 s) transferred to liquid N_2 and then ground in 4 N formic acid in 50% ethanol. Insoluble compounds were removed by centrifugation and product analyses were performed on the supernatant as described (24, 27).

Organelle Separation. Mitochondria were isolated from protoplasts by minor modifications of the procedures of Nishimura *et al.* (20). Plants were held in darkness for 48 h beforehand in order to reduce the copious amounts of starch that accumulated due to the CO_2 enrichment required for growth of the plants. Protoplasts were prepared in high yield by floating leaves from which the lower epidermis was removed on a solution containing 0.5 M sorbitol, 15 mM Mes (pH 5.5), 1 mM $CaCl_2$, 2% Macerase, and 1.3% Cellulysin. Digestion was about 90% complete after 2 h, at which time the protoplasts were collected by low-speed centrifugation (100*g* for 3 min). The protoplasts was washed in the same buffer lacking enzymes and then resuspended in 2 ml 0.33 M sorbitol, 20 mM Hepes (pH 7.5), 2 mM DTT, and 0.2% BSA. Rupture of the protoplasts was accomplished by one slow passage through a 20-μm net of 1.5 cm^2 area. A 1.7-ml aliquot containing 200 μg Chl was applied to a 15-ml linear gradient of sucrose (35–65% w/v), dissolved in 20 mM Hepes (pH 7.5), 2 mM DTT, and 0.2% BSA , and centrifuged in a swinging bucket rotor at 74,000*g* (average) for 3 h. Upon completion of the run, fractions were collected and brought to 0.05% Triton X-100, and aliquots were removed for assay.

Enzyme Assays. Activity of RuBP carboxylase (24), NAD-malate dehydrogenase (1), and fumarase (21) was measured as described. Serine transhydroxymethylase was assayed by the method of Taylor and Weissbach (29) with the following minor modification. Following completion of the Dimedone-formaldehyde reaction, 1 ml water (0 C) was added. The suspension was centrifuged and the supernatant was discarded. The precipitate was suspended in 1 ml water and extracted with toluene as described (29). This modification significatly reduced background counts which othersise might obscure low levels of activity. Tetrahydrofolate was obtained commercially or prepared by chemical reduction of folic acid (5).

Crude extracts were prepared by grinding 200 mg leaf material in 4 ml 30 mM Hepes (pH 7.5), 1 mM DTT, 0.1 mM pyridoxal phosphate, and 0.05% Triton X-100. The extracts were clarified by centrifugation at 30,000*g* for 15 min. Enzyme assays were performed at 22 C. Protein was determined with a dye-binding assay (28).

RESULTS

Labeling Studies. Among the mutants originally recovered on the basis of a growth requirement for high levels of CO_2 (24), several accumulated high levels of [^{14}C]glycine during $^{14}CO_2$ fixation in atmospheres containing O_2. This can be seen by comparing the distribution of label in products resulting from 10 min photosynthesis in $^{14}CO_2$ and 21% O_2 in wild type *Arabidopsis* and the mutant lines CS42 and CS64 (Table I). In the wild type, about 6% of the label accumulated in glycine, whereas, in the mutants, about 47% of the label accumulated in glycine. The accumulation of labeled glycine in the mutants was accompanied by a greatly reduced labeling of serine and a smaller reduction in labeling of the neutral and insoluble (starch) fractions. These results indicated that the mutants were unable to metabolize glycine to serine. The reduced labeling of the carbohydrate pools was similar to results obtained with serine-glyoxylate aminotransferase-deficient (*sat*) mutants in which serine was an end product of photorespiratory carbon flow (25). In both cases, the reduced labeling of carbohydrate is attributed to the absence of recycling of photorespiratory carbon through the terminal steps of the photosynthetic carbon oxidation cycle to 3-phosphoglycerate. Accumulation of carbon in photosynthetic carbon oxidation cycle intermediates apparently reduces the amount of carbon in the Calvin cycle available for carbohydrate synthesis.

Table I. *Products of $^{14}CO_2$ Photoassimilation by Wild-type and Mutant Arabidopsis*

Intact plants were placed in a darkened cuvette and equilibrated with 350 μl l^{-1} CO_2, 21% O_2, balance N_2. At time zero, the light was turned on and $^{14}CO_2$ was introduced. After 10 min, the plants were quickly removed to liquid N_2 and then extracted, and the products were separated. Photosynthesis rates in the mutants and wild type, determined with an IR gas analyzer under identical conditions, increased over the period of the experiment to 57.2 and 58.9 μmol CO_2 mg^{-1} Chl h^{-1}, respectively. Average ^{14}C incorporation per plant was 0.66, 0.69, 0.70 μCi for wild-type, CS42, and CS64, respectively.

Fraction	$^{14}CO_2$ Incorporated		
	Wild-type	CS64	CS42
	% of total		
Basic fraction	25.0	61.3	61.4
Glycine	6.1	48.1	46.9
Serine	9.5	1.9	1.6
Neutral fraction	30.7	14.5	13.6
Acid-1 fraction	21.7	11.6	12.5
Acid-2 fraction	10.0	8.9	8.5
Insoluble	11.5	3.3	2.9
Recovery	98.9	99.6	99.0

Table II. *Serine Transhydroxymethylase and Fumarase Activity in Leaf Extracts of Wild-type and Mutant Arabidopsis*

Source	Serine Transhydroxymethylase Activity	Fumarase Activity
	nmol min^{-1} mg^{-1} protein	
Wild type	2.0	5.8
CS42	0.3	6.2
CS64	0.3	6.7
CS88	0.3	13.6
F_1 (WT × CS42)	1.3	6.7
F_1 (WT × CS64)	1.5	4.9
F_1 (CS64 × CS42)	0.4	5.8
Mixture[a]	1.4	4.4

[a] Equal volumes of wild-type and mutant (CS64) extract were mixed immediately prior to assay.

Biochemical Characterization. The synthesis of serine from glycine is catalyzed by the enzyme serine transhydroxymethylase. Crude extracts of the mutants exhibited 15% of wild type levels of serine transhydroxymethylase activity but showed normal levels of the control mitochondrial enzyme fumarase (Table II). Mixing of mutant and wild type extracts resulted in approximately additive levels of activity (Table II), suggesting that the reduced activity in the mutants was not due to the presence of an inhibitor.

Several lines of evidence suggested that the mutants were totally deficient in mitochondrial serine transhydroxymethylase activity and that the 15% residual activity was due to a second, extrami-

tochondrial enzyme. The glycine pool in the mutants was nearly static during a 20-min $^{12}CO_2$ chase in the dark (Fig. 1A) and actually increased during a 20-min $^{12}CO_2$ chase in the light (Fig. 1B). In contrast, labeled glycine turned over very quickly in the wild type under both conditions (Fig. 1), as was previously observed in similar experiments with other C_3 species (4, 16). Assuming that the glycine remains accessible to the mitochondrion, these observations are inconsistent with the presence of significant amounts of serine transhydroxymethylase activity in the mitochondrion. The stability of the glycine pool also suggested that the glycine decarboxylation reaction was blocked because the glycine was not dissipated by decarboxylation to CO_2.

Additional evidence that the mutants lack mitochondrial seritranshydroxymethylase activity was obtained by cell fractionation. Protoplast lysates were centrifuged on a 35 to 65% sucrose gradient to separate particulate enzyme activities (Fig. 2). Because the chloroplasts were totally disrupted by this procedure, all of the RuBP carboxylase activity remained at the top of the gradient. In contrast to the chloroplast marker, a substantial amount of activity for the mitochondrial marker NAD-malate dehydrogenase entered the gradient in both wild type and mutant preparation. The particulate nature of the malate dehydrogenase activity probably reflects the fact that mitochondrial NAD-malate dehydrogenase is a matrix space enzyme and would be released only upon rupture of both mitochondrial membranes. Mitochondrial serine transhydroxymethylase has also been reported to be located in the matrix space (30) and the distribution of activity for this enzyme in wild-type lysates closely followed malate dehydrogenase activity (Fig. 2A). In contrast, lysates of the mutant CS64 lacked particulate serine transhydroxymethylase activity (Fig. 2B), although they had amounts of particulate malate dehydrogenase activity comparable to that of the wild type. Although we were not able to ascertain the location of the residual serine transhydroxymethylase activity, it was clearly extramitochondrial. The existence of two isozymes of serine transhydroxymethylase in leaves has been suggested previously on the basis of cell-fractionation studies (10, 23, 30) and enzymological analysis (17). Also,

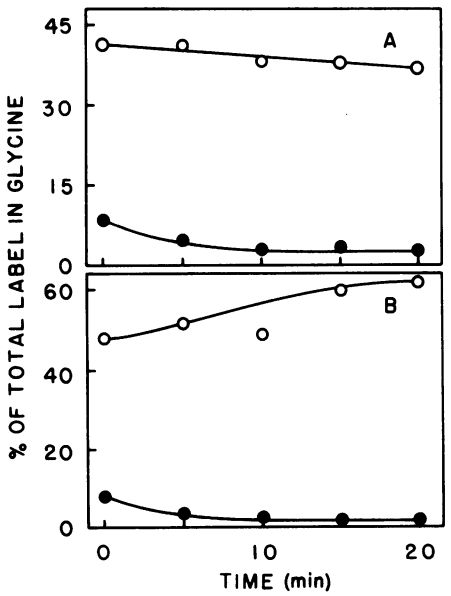

FIG. 1. Persistence of label in glycine pools during a dark (A) or light (B) chase with $^{12}CO_2$. Intact plants were labeled for 10 min with 350 $\mu l\ l^{-1}$ $^{14}CO_2$, 21% O_2, balance N_2; then $^{12}CO_2$ in 21% O_2, balance N_2 was introduced. At the indicated intervals, plants were removed to liquid N_2 and then extracted, and the products were identified. Each point represents the average of two independently treated plants. (●), wild-type; (○), *stm* mutant CS64.

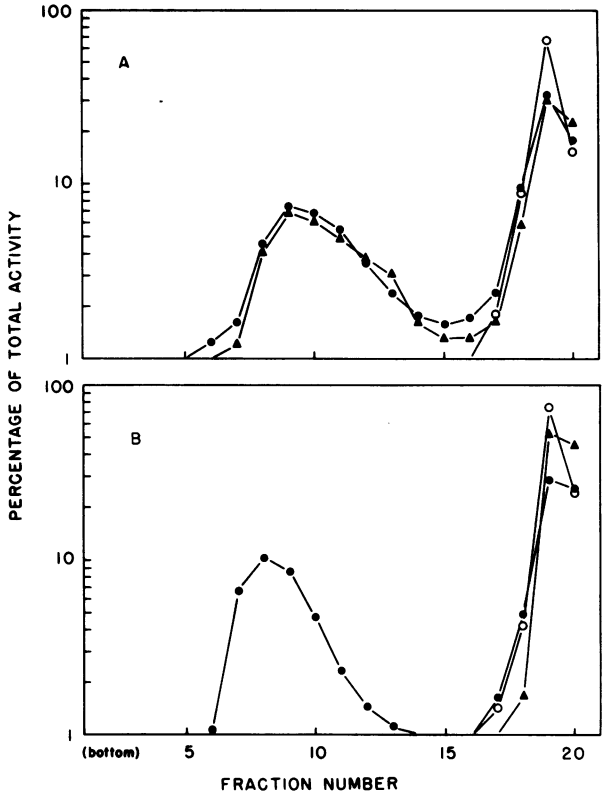

FIG. 2. Distribution of enzyme activities following sucrose gradient centrifugation of ruptured protoplasts of wild-type (A) and mutant (B) *Arabidopsis*. The activity of each fraction is expressed as a percentage of the total activity applied to the gradient. Activities of less than 1% are not presented. (▲), serine transhydroxymethylase; (●), NAD-malate dehydrogenase; (○), RuBP carboxylase.

mutants of yeast have been described which lack mitochondrial serine transhydroxymethylase but contain a second, distinguishable enzyme which is controlled or specified by a separate gene (33).

Genetic Analysis. A single recessive nuclear mutation appears to be responsible for the mutant phenotype. Of 651 F_2 progeny from a WT × CS64 cross, 154 showed the mutant phenotype (requirement for high CO_2) and 497 were of wild-type phenotype (χ^2, 0.62; $P > 0.4$). The F_1 progeny of reciprocal crosses between the mutants and wild type showed the wild-type phenotype and had levels of enzyme activity intermediate to that of the parents (Table II). F_1 progeny from crosses between the mutants had the mutant phenotype and the low level of serine transhydroxymethylase activity characteristic of the mutant parents (Table II). The lack of genetic complementation indicated that the three mutants carry defective alleles of the same gene which we have designated *stm*.

Gas-exchange Analysis. Although the mutants were unable to metabolize glycine, they exhibited a substantial rate of light and O_2-dependent release of CO_2 into a CO_2-free gas stream (Fig. 3). By this criterion, the mutants had a rate of photorespiration which was as much as 30% of that observed in the wild-type. This observation, in conjunction with the results above, suggested that CO_2 was arising by a mechanism unrelated to glycine decarboxylation.

Consideration of the probable sequence of metabolic events which occur in the mutants during illumination under photorespiratory conditions suggested that, inasmuch as glycine is a stable end product of photosynthesis, continued exposure to photorespiratory conditions could result in all readily transferable amino groups becoming trapped in glycine due to continued glyoxylate

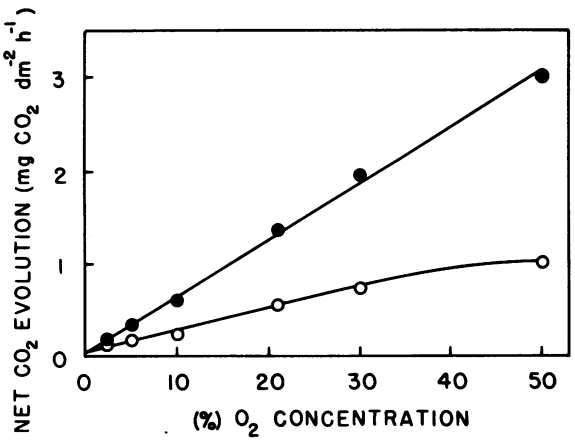

FIG. 3. O_2 response of CO_2-evolution into CO_2-free gas by intact plants of wild-type and mutant *Arabidopsis*. Plants were placed in an illuminated, temperature-controlled cuvette and continuously flushed with a CO_2-free stream of N_2 containing O_2 at the indicated concentration which exited to an IR gas analyzer. Values represent the average steady state rate of CO_2 evolution from measurements made on three plants. The order of exposure to different O_2 concentrations was randomized. (●), wild-type; (○), *stm* mutant CS64.

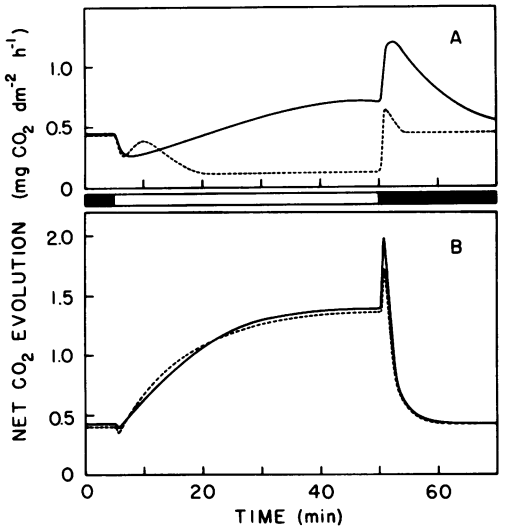

FIG. 4. CO_2 evolution into a CO_2-free gas stream by leaves of mutant (A) and wild-type (B) *Arabidopsis* in the presence or absence of NH_3. (——), response of untreated leaves; (– – –), response of leaves placed on medium containing 10 mM $(NH_4)_2SO_4$. The gas stream contained 50% O_2, 50% N_2. The broken bar between graphs A and B represents light or dark conditions.

synthesis and amination. The NH_3 demand imposed by glyoxylate transamination far exceeds the ability of the plant to provide reduced nitrogen from primary NO_3 reduction (9), so the rate of glycine synthesis would be expected to decline eventually due to depletion of suitable amino donors (glutamate, alanine, serine; see ref. 32). This analysis suggested that the CO_2 released by the mutants was arising from a reaction preceding glycine decarboxylation and that CO_2 release might be overcome by addition of supplementary NH_3.

To test this possibility, gas exchange was measured on leaf fragments provided with exogenous NH_3. The lower epidermis of the leaves was removed to facilitate the uniform uptake of NH_3. Leaf fragments of the wild type released CO_2 into a CO_2-free gas stream (50% O_2, 50% N_2) in a typical response which was unaffected by the presence of 10 mM NH_3 in the medium (Fig. 4B).

Table III. *Soluble Products of $^{14}CO_2$ Assimilation by Leaves of Wild-type and Mutant Arabidopsis in Presence or Absence of Exogenous NH_3*

Leaves with the lower epidermis removed were placed on an agarose gel containing 10 mM $(NH_4)_2SO_4$ as indicated and illuminated for 40 min in 50% O_2, 50% N_2. At this time, $^{14}CO_2$ was introduced to a final concentration of 400 $\mu l\ l^{-1}$. After 7 min incorporation, the leaves were quickly (3–5 s) removed to liquid N_2 and then extracted and the products were identified. Total ^{14}C incorporation averaged 0.65 and 0.25 μCi for wild type and CS42, respectively.

Fraction	$^{14}CO_2$ Incorporated			
	Untreated		NH_3-treated	
	Wild-type	CS42	Wild-type	CS42
	% of total			
Basic fraction	55.8	20.0	35.1	64.2
Glycine	24.8	12.9	12.7	49.4
Serine	22.8	2.2	9.6	1.4
Neutral fraction	6.1	12.5	7.9	4.2
Acid-1 fraction	21.6	27.1	36.1	15.1
Malate	8.1	12.6	20.8	2.9
Glycolate	0.1	0.4	0.4	0.3
Glyoxylate	0	1.9	0	0.1
Acid-2 fraction	11.5	17.3	14.6	5.3
Acid-3 fraction	5.5	24.5	7.7	8.5
FBP[a]	1.4	7.4	2.0	2.9
RuBP	2.4	12.9	3.4	4.6
Recovery	100.5	101.4	101.4	97.3

[a] FBP, fructose 1,6-bisphosphate.

Leaf fragments of the mutant line CS64 released CO_2 at about 50% of wild-type rates when placed on unsupplemented medium but did not show photorespiration when placed on 10 mM NH_3 (Fig. 4A). The low residual level of CO_2 release was not affected by O_2 concentration (results not presented) and was, therefore, probably unrelated to photorespiratory carbon metabolism. Compared to the wild type, both intact plants (results not presented) and detached leaves of the mutant exhibited a relatively large postillumination burst which was nearly eliminated by the provision of NH_3 (Fig. 4). Considered together, these results suggest that the mutants undergo amino depletion under continued photorespiratory conditions. The lack of response by wild-type leaves to exogenous NH_3 indicated that, even under relatively severe photorespiratory conditions (CO_2-free 50% O_2), the rate of internal recycling of NH_3 (9, 26) was sufficient to meet the demand for amide donors for glyoxylate amination.

Labeling of Leaf Fragments. The explanation given above for the gas-exchange data was substantiated by the patterns of product formation following $^{14}CO_2$ incorporation by leaf fragments in the presence or absence of NH_3. Before labeling, leaves were illuminated in a CO_2-free gas stream (50% O_2, 50% N_2) for 40 min to permit glycine accumulation. The results of this experiment (Table III) provided support for the hypothesis of progressive reduction of glycine synthesis due to amino depletion. In the wild type, NH_3 reduced the proportion of label found in amino acids. This was accompanied by a large increase in the proportion of label found in malate. The stimulation of malate labeling by NH_3 is unexplained but has been observed in several other organisms under similar conditions (6). By contrast, in the mutant CS42, the proportion of label which accumulated in amino acids (mainly glycine) was greatly increased in the presence of NH_3. We consider this strong evidence that the flow of carbon to glycine was inhibited in the unsupplemented mutant leaves because of amino depletion. Glycine synthesis was not inhibited immediately following the onset of illumination (Table I) because the endogenous amino supply was presumably adequate to permit flow of carbon

from glyoxylate to glycine during the time period of that experiment.

The elimination of photorespiration by exogenous NH_3 suggested that the general increase of label in the neutral and acid fractions of the untreated mutant and the lack of accumulation of label in photorespiratory organic acids were due to the rapid and quantitative conversion of a glycine precursor, probably glyoxylate, to CO_2. Thus, a portion of label which was trapped in glycine in the NH_3-treated mutant leaves was lost in the untreated leaves, causing an apparent increase in the proportion of label in other compounds.

Photosynthesis Measurements. The effects of the *stm* mutation on photosynthesis were similar to those observed in other mutants deficient in photorespiratory pathway enzymes (24–26). In atmospheric conditions which prevented oxygenation of RuBP, *i.e.* high CO_2 or low O_2, the photosynthetic characteristics of the mutants were indistinguishable from those of the wild-type (results not presented). However, under conditions which promoted photorespiration, photosynthesis was severely impaired. This is apparent by comparing the photosynthetic response of wild-type and mutant (CS64) leaf fragments (Fig. 5). Following illumination in 357 $\mu l\ l^{-1}$ CO_2, 50% O_2, balance N_2, the photosynthesis rate of the mutant initially approached that of the wild-type but rapidly (20 min) declined to about 7% of the wild-type rate. Similar results were observed with intact plants, and all three mutants showed an identical response. As with other mutants of the photorespiratory pathway (24–26), the inhibition of photosynthesis was reversed by a short dark period (Fig. 6). The rapidity of recovery indicated that it was not glycine accumulation *per se* which inhibited photosynthesis inasmuch as the glycine pool remained static during the recovery period (Fig. 1).

Exogenous NH_3 had no significant effect on photosynthesis by the wild-type (Fig. 5B). In contrast, 10 mM NH_3 partially alleviated the O_2 inhibition of photosynthesis observed in the *stm* mutant (Fig. 5A), so that the rate remained at about 40% of wild-type rate. This effect may have been simply due to increased net photosynthesis resulting from the NH_3 elimination of photorespiratory CO_2 release discussed above. This conclusion was supported by the results presented in Figures 4 and 5. The treated and untreated photosynthesis rates were comparable when the observed rate of photorespiration was subtracted from the net photosynthesis rate of the NH_3-treated material.

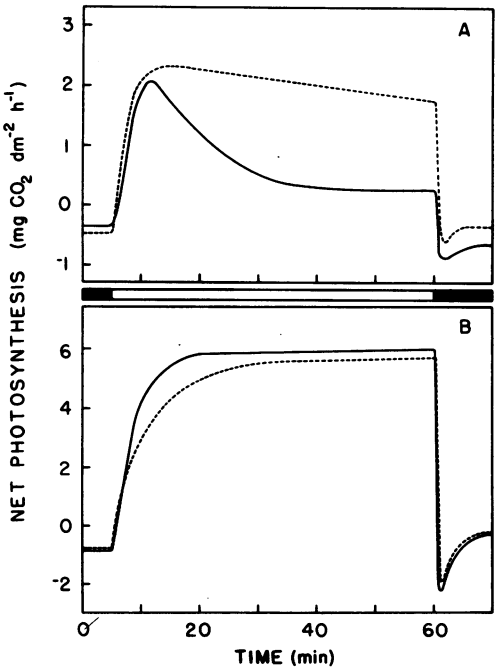

FIG. 5. Net photosynthetic CO_2 fixation by leaves of mutant CS64 (A) and wild-type (B) *Arabidopsis* in the presence (– – –) or absence (——) of 10 mM $(NH_4)_2SO_4$. The gas stream contained 357 $\mu l\ l^{-1}$ CO_2, 50% O_2, balance N_2. The broken bar between graphs A and B represents the conditions of illumination.

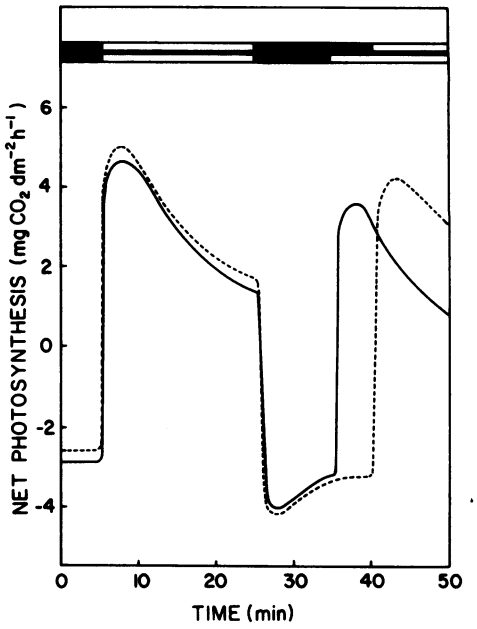

FIG. 6. Dark induced recovery of photosynthetic capacity in an *stm* mutant of *Arabidopsis*. Gas exchange was monitored on intact plants of the mutant CS64 which was continuously flushed with 357 $\mu l\ l^{-1}$ CO_2, 50% O_2, balance N_2. Immediately preceding the experiment the plants were illuminated in 350 $\mu l\ l^{-1}$ CO_2, 2% O_2, balance N_2 until they had achieved maximal rates of photosynthesis. The light then was turned off for 10 min, during which time the system was equilibrated with the 50% O_2 gas regime. The bars represent light or dark conditions.

DISCUSSION

A distinguishing characteristic of the *stm* mutants was an almost complete inability to metabolize photorespiratory glycine. The stability of the glycine pool, the reduced labeling of serine, and the absence of photorespiratory CO_2 evolution under conditions of adequate NH_3 supply indicated that the mutants were defective in both glycine decarboxylation and the glycine to serine conversion. Both of these defects can be attributed to the observed loss of mitochondrial serine transhydroxymethylase activity. In intact mitochondria, this reaction is necessary for the regeneration of THF, the C_1 acceptor in the glycine decarboxylation reaction (13, 19). Because the glycine decarboxylase assay requires intact mitochondria and is dependent upon endogenous serine transhydroxymethylase activity, it is not possible to exclude the possibility that this glycine decarboxylase activity was also missing. There is, however, no reason to suppose this at present. The small burst of photorespiratory CO_2 observed immediately following illumination of NH_3-supplemented leaves of the *stm* mutant (Fig. 4A) probably represented a burst of glycine decarboxylation in which the endogenous pool of the THF was consumed. The physiological, biochemical, and genetic properties of the mutants are attributable to a single nuclear mutation.

Nuclear control of mitochondrial serine transhydroxymethylase in *Arabidopsis* is analogous to the case in yeast where a nuclear gene codes for the mitochondrial isozyme of serine transhydroxymethylase. In yeast, however, the loss of mitochondrial serine transhydroxymethylase activity rendered the organism auxo-

trophic for several compounds (methionine, thymine, adenine, histidine) derived from C_1-THF. That this was not the case in *Arabidopsis* is consistent with a large body of evidence that the chloroplast is the major site of amino acid biosynthesis. It seems likely, as previously suggested (23), that the other isozyme of serine transhydroxymethylase is chloroplastic. The normal growth of the mutants under nonphotorespiratory conditions indicated mitochondrial serine transhydroxymethylase is not required in C_3 plants for any function unrelated to photorespiration.

The absence of photorespiration in leaves of the *stm* mutants supplied with exogenous NH_3 indicated that glycine decarboxylation was the sole site of photorespiratory CO_2 released under conditions of amino supply which permit the uninterrupted flow of photorespiratory carbon to glycine. The availability of amino donors for glycine synthesis did not appear to be limiting in wild-type leaves, even under elevated O_2, because added NH_3 had no effect on the rate of CO_2 evolution. Under normal physiological conditions, glycine decarboxylation appeared to be the sole site of photorespiratory CO_2 release.

It has been suggested (4, 7, 8, 15, 34) that, under some conditions, glyoxylate may be decarboxylated directly by the peroxidatic action of catalase or oxidized to CO_2 and formate nonenzymically. Results presented here suggested that this may occur in the *stm* mutants, but only upon depletion of amino donors for the amination of glyoxylate. First, the existence of NH_3-sensitive photorespiration is consistent with this hypothesis. Second, the sharp reduction in flow of label to glycine in leaves of the *stm* mutants after prolonged exposure to photorespiratory conditions is consistent with diversion of glycolate carbon to another CO_2-evolving mechanism. The absence of significant accumulation of label in any of the photorespiratory organic acids precluded demonstration of a particular reaction and suggested that, if glyoxylate decarboxylation is the CO_2 source, the decarboxylation reaction is very rapid. Further analysis is required to determine the precise mechanism.

The effect of NH_3 supplementation on net photosynthetic CO_2 fixation by the mutants seems to be adequately explained by NH_3 suppression of photorespiratory CO_2 release from organic acids. However, inasmuch as NH_3 did not completely restore photosynthetic capacity in the mutants, it seems that additional factors are involved in the inhibition. We have previously suggested, in the case of mutants deficient in serine-glyoxylate aminotransferase (25), that inhibition was due in part to lack of recycling of photorespiratory carbon through the terminal steps of photorespiratory pathway. This explanation may also be applicable to the *stm* mutants described here.

Acknowledgments—We thank S. C. Somerville, M. H. Spalding, and D. T. Canvin for helpful discussion.

LITERATURE CITED

1. Asahi T, M Nishimura 1973 Regulatory function of malate dehydrogenase isoenzymes in the cotyledons of mung bean. J Biochem 73: 217–225
2. Atkins CA, DT Canvin 1971 Photosynthesis and CO_2 evolution by leaf discs: gas exchange, extraction, and ion exchange fractionation of ^{14}C-labeled photosynthetic products. Can J Bot 49: 1225–1234
3. Bird IF, MJ Cornelius, AJ Keys, CP Whittingham 1972 Oxidation and phosphorylation associated with the conversion of glycine to serine. Phytochemistry 11: 1587–1594
4. Canvin DT, NDH Lloyd, H Fock, K Przybylla 1976 Glycine and serine metabolism and photorespiration. *In* RH Burris, CC Black, eds, CO_2 Metabolism and Plant Productivity. University Park Press, Baltimore, pp 161–176
5. Davis L 1968 A simple method for the synthesis of tetrahydrofolic acid. Anal Biochem 26: 459–460
6. Graham D, EA Chapman 1979 Interactions between photosynthesis and respiration in higher plants. *In* M Gibbs, E Latzko, eds, Encyclopedia of Plant Physiology, New Series, Vol 6. Springer-Verlag, New York, pp 150–160
7. Grodzinski B 1978 Glyoxylate decarboxylation during photorespiration. Planta 144: 31–37
8. Halliwell B, VS Butt 1974 Oxidative decarboxylation of glycollate and glyoxylate by leaf peroxisomes. Biochem J 138: 217–224
9. Keys AJ, IF Bird, MJ Cornelius, PJ Lea, RM Wallsgrove, BJ Miflin 1978 Photorespiratory nitrogen cycle. Nature 275: 741–743
10. Kisaki T, A Imai, NE Tolbert 1971 Intracellular localization of enzymes related to photorespiration in green leaves. Plant Cell Physiol 12: 267–273
11. Kisaki T, NE Tolbert 1970 Glycine as a substrate for photorespiration. Plant Cell Physiol 11: 247–258
12. Kisaki T, N Yoshida, A Imai 1971 Glycine decarboxylase and serine formation in spinach leaf mitochondrial preparations with reference to photorespiration. Plant Cell Physiol 12: 275–288
13. Klein SM, RD Sagers 1967 Glycine metabolism. III. A flavin-linked dehydrogenase associated with the glycine cleavage system in *Peptococcus glycinophilus*. J Biol Chem 242: 297–300
14. Kumarasinghe KS, AJ Keys, CP Whittingham 1977 Effects of certain inhibitors on photorespiration by wheat leaf segments. J Exp Bot 28: 1163–1168
15. Lawyer AL, I Zelitch 1979 Inhibition of glycine decarboxylation and serine formation in tobacco by glycine hydroxamate and its effect on photorespiratory carbon flow. Plant Physiol 64: 706–711
16. Mahon JD, H Fock, DT Canvin 1974 Changes in specific radioactivity of sunflower leaf metabolites during photosynthesis in $^{14}CO_2$ and $^{12}CO_2$ at three concentrations of CO_2. Planta 120: 245–254
17. Mazelis M, ES Liu 1967 Serine transhydroxymethylase of cauliflower: partial purification and properties. Plant Physiol 42: 1763–1768
18. Moore AL, C Jackson, B Halliwell, JE Dench, DO Hall 1977 Intramitochondrial localisation of glycine decarboxylase in spinach leaves. Biochem Biophys Res Commun 78: 483–491
19. Motokawa Y, G Kikuchi 1974 Glycine metabolism by rat liver mitochondria. Arch Biochem Biophys 164: 624–633
20. Nishimura M, D Graham, T Akazawa 1976 Isolation of intact chloroplasts and other cell organelles from spinach leaf protoplasts. Plant Physiol 58: 309–314
21. Racker E 1950 Spectrophotometric measurements of the enzymatic formation of fumaric and cis-aconitic acids. Biochim Biophys Acta 4: 211–214
22. Rédei GP 1970 *Arabidopsis thaliana* (L.) Heyn. A review of the genetics and biology. Bibliogr Genet 20: 1–151
23. Shah SPJ, EA Cossins 1970 The biosynthesis of glycine and serine by isolated chloroplasts. Phytochemistry 9: 1545–1551
24. Somerville CR, WL Ogren 1979 A phosphoglycolate phosphatase deficient mutant of *Arabidopsis*. Nature 280: 833–836
25. Somerville CR, WL Ogren 1980 Photorespiration mutants of *Arabidopsis thaliana* deficient in serine-glyoxylate aminotransferase activity. Proc Natl Acad Sci USA 77: 2684–2687
26. Somerville CR, WL Ogren 1980 Inhibition of photosynthesis in mutants of *Arabidopsis* lacking glutamate synthase activity. Nature 286: 257–259
27. Somerville CR, WL Ogren 1981 Isolation of photorespiration mutants of *Arabidopsis*. *In* M Edelman, RB Hallick, NH Chua, eds, Methods in Chloroplast Molecular Biology. Elsevier, Amsterdam. In press
28. Spector T 1978 Refinement of the Coomassie blue method of protein quantitation. Anal Biochem 86: 142–146
29. Taylor RT, H Weissbach 1968 Radioactive assay for serine transhydroxymethylase. Anal Biochem 13: 80–84
30. Woo KC 1979 Properties and intramitochondrial localization of serine hydroxymethyltransferase in leaves of higher plants. Plant Physiol 63: 783–787
31. Woo KC, CB Osmond 1976 Glycine decarboxylation in mitochondria isolated from spinach leaves. Aust J Plant Physiol 3: 771–785
32. Yamazaki RK, NE Tolbert 1970 Enzymic characterization of leaf peroxisomes. J Biol Chem 245: 5137–5144
33. Zelikson R, M Luzzati 1976 Two forms of serine transhydroxymethylase, one absent in a thymidylate-less mutant in *Saccharomyces cerevisiae*. Eur J Biochem 64: 7–13
34. Zelitch I 1972 Comparison of the effectiveness of glycolic acid and glycine as substrates for photorespiration. Plant Physiol 50: 109–113.