Plant Physiol. (1979) 63, 783–787
0032-0889/79/63/0783/05/$00.50/0

Properties and Intramitochondrial Localization of Serine Hydroxymethyltransferase in Leaves of Higher Plants

Received for publication July 12, 1978 and in revised form November 4, 1978

K. C. Woo
Department of Biology, Queen's University, Kingston, Ontario K7L 3N6 Canada

ABSTRACT

The activity of serine hydroxymethyltransferase in mitochondria isolated from spinach leaves was absolutely dependent on tetrahydrofolate; pyridoxal phosphate has no effect on the activity. The stability of this activity in the isolated mitochondria was dependent on the presence of sulfhydryl compounds. It was apparently more stable at pH 7.0 to 7.5 than at higher pH even though the pH optimum of serine hydroxymethyltransferase was 8.5 for both the mitochondrial and cytoplasmic fractions. Distribution studies have indicated that serine hydroxymethyltransferase was predominantly located in the mitochondria. The activity of serine hydroxymethyltransferase was observed to be co-compartmented with glycine decarboxylation and malate dehydrogenase behind the mitochondrial inner membrane. This activity could be solubilized by KCl from osmotically ruptured mitochondrial membrane fractions but substantial activity (35 to 40%) was still retained with the membrane fractions at 0.3 M KCl. This suggests that the glycine decarboxylation-serine hydroxymethyltransferase complex may be closely bound to the internal surface of the mitochondrial inner membrane.

The relationship of this integrated enzyme complex to CO_2 evolution and serine synthesis during photorespiration and the physiological role of the dicarboxylate shuttle were discussed.

The CO_2 released during photorespiration is derived principally from the decarboxylation of glycine in leaf mitochondria (4, 32, 33). This glycine decarboxylation is coupled to serine synthesis through the activity of serine hydroxymethyltransferase (2, 13). The stoichiometry of the reaction for glycine decarboxylation and serine synthesis in isolated leaf mitochondria suggests that the activity of serine hydroxymethyltransferase is equivalent to the rate of glycine decarboxylation. We have reported rates of serine hydroxymethyltransferase in isolated leaf mitochondria of C_3 plants comparable to those of glycine decarboxylation (15).

The activity of serine hydroxymethyltransferase in nonphotosynthetic (29) and photosynthetic (6, 21) tissues of higher plants has been reported but the intracellular localization of this enzyme in these tissues is not known, although this enzyme is reported to be present in pea chloroplasts (26). Recent studies with rat liver (20) and mutants of *Saccharomyces cerevisiae* (34) have revealed the presence of cytoplasmic and mitochondrial alloenzymes of serine hydroxymethyltransferase. In rat liver mitochondria the enzyme is located within the matrix (7). Earlier studies have indicated localization of glycine decarboxylation activity behind the inner membrane of the mitochondria (33). This would also imply the localization of serine hydroxymethyltransferase in this compartment.

We have examined the properties of this mitochondrial serine hydroxymethyltransferase from spinach leaves and provided evidence for its co-compartmentation with glycine decarboxylation activity behind the inner membrane of the mitochondria.

MATERIALS AND METHODS

Preparation of Mitochondria from Spinach Leaves. Spinach leaves (20 g) were deribbed and blended for 2 × 5 s in 100 ml of a medium containing 0.4 M sucrose, 0.1 M Hepes-NaOH buffer (pH 7.5), 10 mM DTT, 2 mM $MgCl_2$, 2 mM EGTA, and 0.1% (w/v) BSA in a Waring Blendor. The homogenate was filtered through two layers of Miracloth and centrifuged at 2,000*g* for 10 min. The pellet was resuspended in 20 ml of 20 mM K-phosphate (pH 7.5) and 1 mM DTT and used as the chloroplast fraction. The crude mitochondrial fraction may be prepared as follows.

(a) For standard serine hydroxymethyltransferase assay the 2,000*g* supernatant was centrifuged at 10,000*g* for 10 min and the pellet was resuspended in 20 ml of 20 mM K-phosphate (pH 7.5) and 1 mM DTT. This fraction was solubilized with 0.05% (w/v) Triton X-100, incubated at 0 C for 10 min, and used as the crude mitochondrial fraction.

(b) For localization studies, the 2,000*g* supernatant was centrifuged at 10,000*g* for 10 min. The pellet was resuspended in 25 ml of a medium containing 0.4 M sucrose, 0.1 M Hepes-NaOH buffer (pH 7.5), 5 mM K-phosphate, 5 mM $MgCl_2$, and 6 mg BSA, and used as the mitochondrial fraction.

(c) For KCl solubilization studies, the 2,000*g* supernatant was centrifuged at 10,000*g* for 10 min. The pellet was resuspended in 2 ml of a medium containing 50 mM sucrose, 12.5 mM Hepes-NaOH buffer (pH 7.5), 1 mM DTT, and 0.1% (w/v) BSA and used as the mitochondrial fraction.

For glycine decarboxylation studies mitochondria were prepared as described (33). The isolation of mitochondrial fractions was carried out at 4 C and chilled solutions and apparatus were used.

Enzyme Assays. The assay for serine hydroxymethyltransferase (at pH 8.5) was based on the procedure of Taylor and Weissbach (27). The activity of NAD-malate dehydrogenase, catalase, fumarase, antimycin A-sensitive NADH-Cyt *c* reductase and glycine decarboxylation was assayed as described in Woo and Osmond (33). RuBP Case[1] activity was assayed as described in Quebedeaux and Chollet (23).

All enzyme activity in the crude mitochondrial fraction was expressed on the basis of the Chl content of the original crude homogenate.

RESULTS AND DISCUSSION

Serine Hydroxymethyltransferase in Leaves of Higher Plants. The distributions of RuBP Case, catalase, and Chl in the homogenate fraction of spinach leaves are shown in Table I. The activities of RuBP Case and catalase are used as enzyme markers for intact chloroplasts and peroxisomes, respectively. The Chl distribution indicates that practically all of the Chl is located in the chloroplast (2,000*g*) fraction. As indicated by the activity of RuBP Case, only

[1] Abbreviations: RuBP Case: ribulose bisphosphate carboxylase; OAA: oxaloacetate.

about 9% of intact chloroplasts are recovered in this fraction. This chloroplast fraction was contaminated with about 20% of the total catalase activity, and about 22% of fumarase activity recovered (Table II). The mitochondrial (20,000*g*) fraction contains less than 1% of the total RuBP Case activity, 3% of the total Chl, and 7% of the total catalase activity. In contrast to the high fumarase activity recovered (about 50%) (Table II), the above data suggest that there is very little contamination by chloroplast enzymes in the mitochondrial fractions.

The intracellular activity of serine hydroxymethyltransferase and fumarase in leaves of three C_3 species is shown in Table II. About 50% of the activity of serine hydroxymethyltransferase present in leaves of these three species is located in the mitochondria. The proportion of fumarase and serine hydroxymethyltransferase activity found in the chloroplast and supernatant fraction was practically the same in each experiment, indicating that breakage of mitochondria during isolation may be responsible for this distribution of enzyme activity. Similar results were routinely obtained in experiments with the 10,000*g* mitochondrial fraction from spinach leaves (data not shown). These results suggest that serine hydroxymethyltransferase is almost exclusively a mitochondrial enzyme in leaves of C_3 plants.

Serine hydroxymethyltransferase was reported to be present in chloroplasts of pea leaves (26) and would presumably be involved in the regeneration of acetyl-CoA for terpenoid biosynthesis in these organelles (10). The reported activity of this chloroplast enzyme is very low in comparison to that of the mitochondrial enzyme reported in Table II. The total activity of serine hydroxymethyltransferase present in the leaf homogenates in Table II would be expected to be derived principally from the mitochondrial enzyme.

General Properties of Serine Hydroxymethyltransferase. The activity of serine hydroxymethyltransferase was linear for the period of the assay and was proportional to the amount of enzyme added. The cofactor requirements for the enzyme in both the crude homogenate and mitochondrial fractions are shown in Table III. The results obtained for both fractions are similar and both show an absolute requirement for tetrahydrofolate. No requirement for pyridoxal phosphate on serine hydroxymethyltransferase activity was observed in the mitochondrial fractions (Table IV).

These results are similar to the results observed for the enzyme from wheat leaf (6) but the enzyme from cauliflower (16), maize (9) and turnip (29) showed marked stimulation by pyridoxal phosphate as well as an absolute requirement for tetrahydrofolate. On the other hand, the enzyme from tobacco roots did not respond to pyridoxal phosphate until treated with KCN or semicarbazide (22). There seem to be considerable differences among the different plant enzymes in cofactor requirements. Alternatively, the lack of stimulation by pyridoxal phosphate might simply reflect a tight binding of the coenzyme to the enzyme during isolation.

The stability of the enzyme is dependent on the presence of sulfhydryl compounds (Fig. 1). Activity is rapidly lost when the enzyme is stored in ice in the absence of either DTT or 2-mercaptoethanol. Greater stability in the presence of DTT is obtained when the enzyme is stored at pH 7.0 to 7.5 compared to storage at higher pH (Table V).

Figure 2 shows the activity of serine hydroxymethyltransferase as a function of pH. All of the different fractions—crude homogenate, mitochondrial fraction (solubilized and unsolubilized), and supernatant fraction—show an optimum at pH 8.5. In contrast, the enzyme from cauliflower buds shows 2 pH optima at 7.5 and 9.5 (16). The cytoplasmic and mitochondrial alloenzymes from rat

Table I. Distribution of ribulose bisphosphate carboxylase (RuBP C'ase), catalase and chlorophyll in spinach leaf extract.

All assays were determined by standard procedure described in Materials and Methods. The chloroplast fraction was prepared as a 2,000 x g pellet fraction. The mitochondrial fraction was prepared as a 20,000 x g pellet fraction instead of the normal 10,000 x g fraction. Numbers in brackets are percent activity.

	Activity (μmole/min)		
Fraction	RuBP C'ase	Catalase	Chlorophyll (μg)
Homogenate	128 (100)	2300 (100)	13070 (100)
Chloroplast	10.9 (9)	464 (20)	13240 (101)
Mitochondria	0.8 (<1)	158 (7)	333 (3)
Supernatant	111.7 (87)	1579 (69)	113 (1)

Table II. Distribution of serine hydroxymethyltransferase and fumarase in leaf extracts of C_3 species.

All assays were determined by standard procedure described in Materials and Methods. The mitochondrial fraction was prepared as a 20,000g pellet fraction instead of the normal 10,000g pellet fraction.

		Specific activity		Total activity			
		SMT*	Fumarase	SMT	Fumarase	% Activity	
		(μmol h^{-1} $mg\ chl^{-1}$)		(μmol h^{-1})			
Species	Fraction					SMT	Fumarase
Spinach	Homogenate	48.3	31.7	422	277	100	100
	Chloroplasts	8.5	6.9	74	60	18	22
	Mitochondria	26.0	16.3	227	142	54	51
	Supernatant	8.4	6.8	73	59	17	21
Vicia faba	Homogenate	28.7	25.0	217	188	100	100
	Chloroplasts	6.4	6.2	49	47	22	25
	Mitochondria	15.7	13.9	119	106	55	56
	Supernatant	5.9	5.8	44	44	21	23
Atriplex hastata	Homogenate	39.9	20.9	277	144	100	100
	Chloroplasts	6.5	4.3	45	30	16	21
	Mitochondria	15.8	9.8	109	68	40	47
	Supernatant	-	5.2	-	36	-	25

*SMT - serine hydroxymethyltransferase.

Table III. Cofactor requirements for serine hydroxymethyltransferase in crude extracts and mitochondria isolated from spinach leaves.

Assay conditions : Standard assay as described in Materials and Methods except 20 mM K phosphate buffer (pH 7.5) was used instead of the standard 50 mM Tris-HCl buffer (pH 8.5); PLP was 2 mM when present. The crude homogenate fractions contained 39 µg chl.

Cofactor	Substrate converted (µmol h^{-1} mg chl^{-1})	
	Homogenate	Mitochondria
None	0.86	0.14
PLP*	0.22	0.04
PLP, FH_4 (0.4 mM)	-	2.58
PLP, FH_4 (2 mM)	18.31	6.67
PLP, FH_4 (4 mM)	-	5.97
FH_4 (2 mM)	19.57	7.23

*PLP, pyridoxal phosphate ; FH_4, tetrahydrofolate.

Table IV. Effect of pyridoxal phosphate on serine hydroxymethyltransferase activity in mitochondria isolated from spinach leaves.

Assay conditions as in Table III with 2 mM FH_4. Mitochondrial fractions, equivalent to a crude homogenate fraction containing 132 µg chl, were used.

Pyridoxal phosphate (mM)	Substrate converted (µmol h^{-1} mg chl^{-1})
0	7.2
0.08	7.1
0.4	6.7
0.8	6.9
2.0	7.2

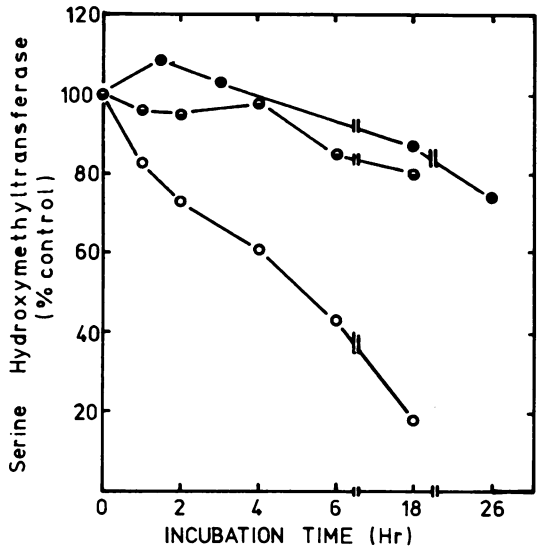

FIG. 1. Stability of serine hydroxymethyltransferase in presence of sulfhydryl compounds. ○: control; ◒: 2-mercaptoethanol (5 mM); ●: DTT (1 mM). Activity of serine hydroxymethyltransferase at zero time in control, in the presence of 2-mercaptoethanol and DTT was 10.6, 10.8, and 10.8 µmol h^{-1} mg Chl^{-1}, respectively. Mitochondrial fractions equivalent to a crude homogenate containing 55 µg Chl were used.

liver show a pH optimum at 8.0 and 7.0 to 7.7, respectively (20). Similarly, the cytoplasmic alloenzyme in yeast, *S. cerevisiae*, has a pH optimum at 8.5 while the mitochondrial alloenzyme shows a pH optimum at around 7.5 when freshly prepared but shifts to pH 8.5 to 9.0 on "aging" (34).

Intramitochondrial Location of Serine Hydroxymethyltransferase and the Solubilization of This Activity by KCl. The location of serine hydroxymethyltransferase was determined by the selective removal of the outer membrane with Triton X-100. The rupture of the outer membrane is indicated by a rapid increase in activity of antimycin A-sensitive NADH-Cyt *c* reductase at 0.008% Triton concentration (Fig. 3a). As the Triton concentration is increased to 0.03% rupture of the inner membrane occurs, indicated by a rapid increase in activity of malate dehydrogenase, a known matrix space enzyme. The activity of serine hydroxymethyltransferase closely follows that of malate dehydrogenase and increases only when the inner membrane is ruptured (Fig. 3b). As expected, the loss of activity of the OAA-stimulated glycine decarboxylation coincides with the rupture of the inner membrane and the solubilization of serine hydroxymethyltransferase and malate dehydrogenase (Fig. 3, a and b). The location of serine hydroxymethyltransferase behind the mitochondrial inner membrane is therefore consistent with the earlier observation (33) that glycine decarboxylation is also located behind the mitochondrial inner membrane.

The relationship of these two enzymes in the mitochondria was further investigated by examining the activity of these enzymes in relation to KCl solubilization. Treatment with KCl increases the activity of both serine hydroxymethyltransferase and malate dehydrogenase recovered in the supernatant fractions (Fig. 4). Maximum recovery was achieved at 0.1 M KCl for serine hydroxymethyltransferase and at 0.05 M for malate dehydrogenase. The increase in activity in the supernatant corresponds to a decrease in the activity recovered in the membrane fractions. The activity of glycine decarboxylation recovered in the membrane fractions is very low since this activity is dependent on mitochondrial membrane integrity (32). The solubilization of serine hydroxymethyltransferase activity observed is similar to that obtained for citrate synthetase and malate synthetase in the glyoxysomes from castor bean endosperm, which are considered to be tightly bound to the membrane compared to malate dehydrogenase (11). Unlike these two synthetases, a substantial proportion (32–38%) of the serine hydroxymethyltransferase activity is retained in the membrane fraction even at high concentration of KCl. This evidence suggests that serine hydroxymethyltransferase is clearly located in the matrix space and fairly tightly bound to the internal surface of the mitochondrial inner membrane.

Table V. Stability of serine hydroxymethyltransferase in the presence of dithiothreitol (1 mM) at different pH.
Activity of serine hydroxymethyltransferase at zero h was 10.8,10.0,14.5,15.6 and 13.7 μmol /h /mg chl for pH 7.0,7.5,8.0, 8.5 (50 mM Tris-HCl) and 8.5 (5 mM Tris-HCl) respectively. Mitochondrial fractions, equivalent to a crude homogenate containing 33 μg chl, were used.

		Substrate converted (% activity)				
		Storage Time (h)				
Buffer	pH	0	1.5	3	19	26	43
50 mM HEPES	7.0	100	113	96	101	104	82
"	7.5	100	118	109	110	107	87
50 mM Tris-HCl	8.0	100	116	101	81	71	52
"	8.5	100	110	87	67	59	45
5 mM Tris-HCl	8.5	100	119	103	87	74	53

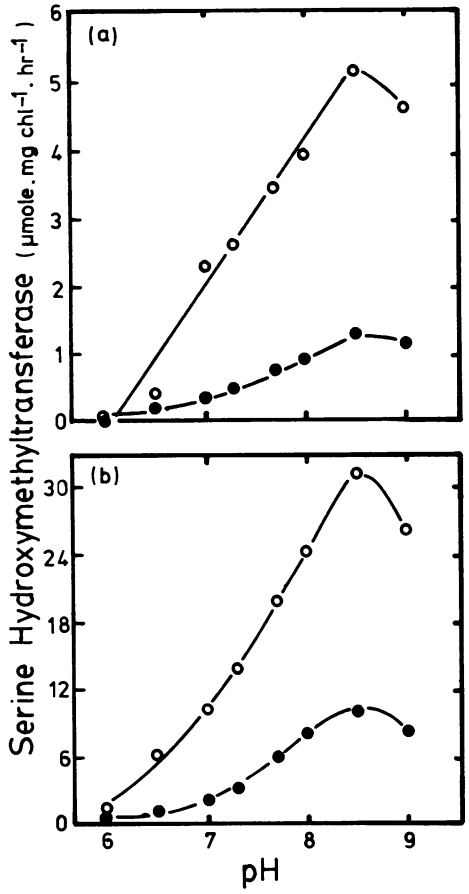

FIG. 2. Activity of serine hydroxymethyltransferase as a function of pH. Assay conditions were as described under "Materials and Methods" except 50 mM Hepes-NaOH buffer was present in addition to the standard 50 mM Tris-HCl buffer used. (a) ●: intact mitochondria; ○: supernatant. (b) ●: solubilized mitochondrial fraction; ○: crude homogenate fraction.

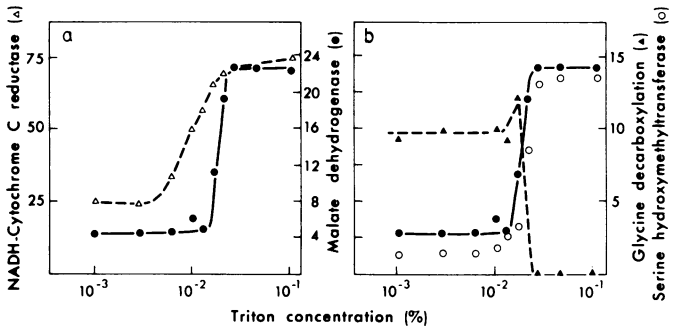

FIG. 3. Effect of Triton X-100 on activity of mitochondrial enzymes. ▲: OAA-dependent glycine decarboxylation; ○: serine hydroxymethyltransferase; △: NADH-Cyt *c* reductase; ●: malate dehydrogenase. Aliquots (2 ml) of crude mitochondrial fraction were solubilized with appropriate concentrations of Triton X-100 and kept at room temperature for 10 min. This fraction was assayed for NADH-Cyt *c* reductase activity. This fraction was centrifuged at 30,000*g* for 10 min. The pellet fraction was assayed for glycine decarboxylation activity, and the supernatant fraction was assayed for malate dehydrogenase and serine hydroxymethyltransferase activity.

CONCLUSION

The properties of serine hydroxymethyltransferase from spinach leaf mitochondria appears to be generally similar to those of the alloenzyme in pea chloroplasts (26) and the enzymes from other plant tissues (6, 16, 22). The above evidence also suggests that in spinach leaves, as is the case in pea cotyledons (5), but not in rat liver (20) or yeast cells (34) the activity of serine hydroxymethyltransferase was predominantly located in the mitochondria. The similarity of the cofactor requirements, kinetic properties, and pH optima of the enzyme from different fractions argues against the presence of significantly different alloenzymes in this tissue.

Glycine decarboxylation is linked to serine hydroxymethyltransferase for serine synthesis. The activity of serine hydroxymethyltransferase in isolated leaf mitochondria of C_3 species appears to be comparable to the rate of glycine decarboxylation (15). The integrated enzyme system of glycine decarboxylation and serine hydroxymethyltransferase appears to be adequate to account for the rate of photorespiration and serine synthesis during photorespiration (4). The organization of this integrated enzyme system in spinach leaf mitochondria is presumably similar to the extensively studied systems in bacteria (14, 24) and rat liver mitochondria (18, 19). The integration of this enzyme system *in vivo* would presumably favor the transfer of carbon from glycine to serine during photorespiration since there is little evidence that the synthesis of glycine from serine could take place *in vivo* even though the reaction of serine hydroxymethyltransferase is readily reversible *in vitro* (28).

Ammonia is released during the decarboxylation of glycine in leaf mitochondria (13, 31). Evidence indicates this photorespiratory NH_3 is refixed outside the mitochondria via glutamine synthetase and glutamate synthase in the chloroplast and the glutamate formed is returned to the peroxisomes for transamination (12, 15, 30, 31). A photorespiratory nitrogen (NH_3) cycle involving the peroxisomes, mitochondria, and chloroplasts is an integral part of the tightly integrated (and stoichiometrically balanced) pathway of photosynthesis and photorespiration (15). In such a tightly integrated system it is most unlikely that the reverse synthesis of glycine from serine could take place in the mitochondria during photosynthesis and photorespiration.

Localization studies have indicated that both the glycine-decarboxylating system and serine hydroxymethyltransferase are located behind the mitochondrial inner membrane. This is consistent with our earlier suggestion (32) that the regeneration of NAD^+ required for glycine decarboxylation could involve either the mitochondrial electron transport chain or the faster matrix malate dehydrogenase.

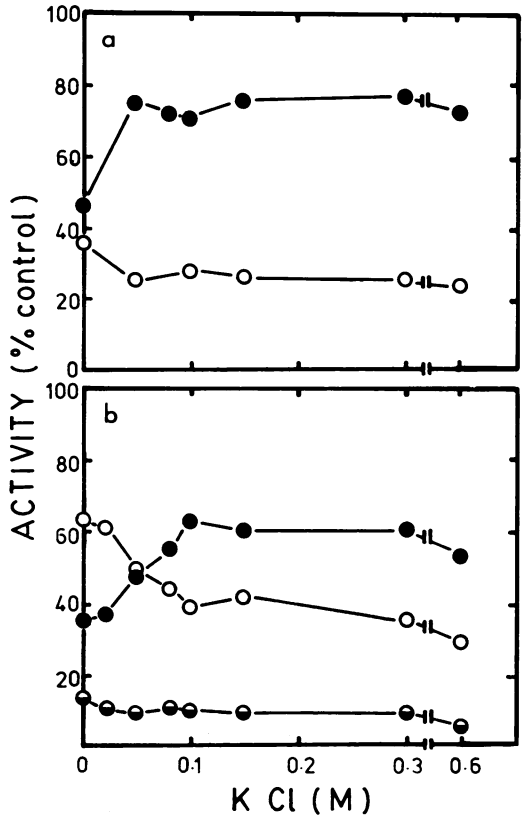

FIG. 4. Solubilization of mitochondrial enzymes with increasing concentration of KCl. (a) (malate dehydrogenase) ○: membrane fraction; ●: supernatant fraction. (b) (serine hydroxymethyltransferase) ○: membrane fraction; ●: supernatant fraction; ◒: OAA-stimulated glycine decarboxylation. Aliquots (50 μl) of crude mitochondrial fraction were pipetted into 4 ml of 5 mM Hepes (pH 7.5) and 1 mM DTT and incubated at 0 C for 15 min. Appropriate KCl concentrations were then added and incubated for an additional 30 min, centrifuged at 30,000*g* for 10 min, and the pellet fraction was assayed for glycine decarboxylation. For malate dehydrogenase and serine hydroxymethyltransferase activity, the pellet and supernatant fractions were solubilized with 0.05% Triton X-100 before assay. All activity is expressed as percentage of control which is the Triton X-100 (0.05%) treated and unfractionated fraction at zero KCl; in the case of glycine decarboxylation the control is the intact mitochondrial fraction at zero KCl.

Recent studies in isolated leaf mitochondria (8, 17) have shown that the oxidation of glycine involving the mitochondrial electron transport chain is, as expected, coupled to the synthesis of ATP. The capacity of this system remains to be reconciled with the *in vivo* rates of photorespiration. If this is indeed the system by which *in vivo* glycine decarboxylation is coupled then it is reasonable to expect mitochondrial electron transport to be unaffected in the light even though tricarboxylic acid cycle metabolism may be affected under these conditions. Recent studies in nitrate reduction (1, 3, 25) in leaves of higher plants had indicated that the normal respiratory electron transport chain may be inhibited or shut down in the light for the reduction of nitrate. This would impose a restriction on glycine decarboxylation coupled to the normal respiratory chain but would not affect glycine decarboxylation linked to a dicarboxylate shuttle in which the NAD^+ requirement for glycine decarboxylation in the mitochondria is coupled to the NADH requirement for hydroxypyruvate reductase in the peroxisomes via a malate-OAA shuttle as proposed by Woo and Osmond (32). In addition, the higher activity observed for this dicarboxylate linked glycine decarboxylation compared to glycine decarboxylation linked to the respiratory electron transport chain (32) may also indicate an *in vivo* role for this dicarboxylate-linked glycine-decarboxylating system during photorespiration.

Acknowledgment—The author thanks Dr. C. B. Osmond for his comments during the preparation of the manuscript.

LITERATURE CITED

1. ATKINS CA, DT CANVIN 1975 Nitrate, nitrite and ammonia assimilation by leaves: effects of inhibitors. Planta 123: 41–51
2. BIRD IF, MJ CORNELIUS, AJ KEYS, CP WHITTINGHAM 1972 Oxidation and phosphorylation associated with the conversion of glycine to serine. Phytochemistry 11: 1587–1594
3. CANVIN DT, CA ATKINS 1974 Nitrate, nitrite and ammonia assimilation by leaves: effect of light, carbon dioxide and oxygen. Planta 116: 207–224
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. CLANDININ MT, EA COSSINS 1972 Localization and interconversion of tetrahydropteroylglutamates in isolated pea mitochondria. Biochem J 128: 29–40
6. COSSINS EA, SK SINHA 1966 The interconversion of glycine and serine by plant tissue extracts. Biochem J 101: 542–549
7. CYBULSKI RL, RR FISHER 1976 Intramitochondrial localization and proposed metabolic significance of serine hydroxymethyltransferase. Biochemistry 15: 3183–3187
8. DOUCE, R, AL MOORE, M NEUBURGER 1977 Isolation and oxidative properties of intact mitochondria isolated from spinach leaves. Plant Physiol 60: 625–628
9. HAUSCHILD HH 1959 The interconversion of glycine and serine in *Zea mays*. Can J Biochem Physiol 37: 887–894
10. HILL HM, SPJ SHAH, LJ ROGERS 1970 Incorporation of [2-^{14}C]glyoxylate, [2-^{14}C]acetate and [2-^{14}C]mevalonic acid into terpenoids during ripening of tomato fruit. Phytochemistry 9: 749–756
11. HUANG AHC, H BEEVERS 1973 Localization of enzyme within microbodies. J Cell Biol 58: 379–389
12. KEYS AJ, IF BIRD, MJ CORNELIUS, PJ LEA, RM WALLSGROVE, BJ MIFLIN 1978 The photorespiratory nitrogen cycle. Nature 275: 741–743
13. KISAKI T, N YOSHIDA, A IMAI 1971 Glycine decarboxylase and serine formation in spinach leaf mitochondrial preparation with reference to photorespiration. Plant Cell Physiol 12: 275–288
14. KOCHI H, G KIKUCHI 1974 Mechanism of the reversible glycine cleavage reaction in *Arthrobacter globiformis*. I. Purification and function of protein components required for the reaction. J Biochem 75: 1113–1127
15. LORIMER GH, KC WOO, JA BERRY, CB OSMOND 1978 The C_2 photorespiratory carbon oxidation cycle in leaves of higher plants: pathway and consequences. *In* DO Hall, J Coombs, TW Goodwin, eds, Proc 4th Int Congr Photosyn. Biochemical Soc, London, pp 311–322
16. MAZELIS M, ES LIU 1967 Serine transhydroxymethylase of cauliflower (*Brassica oleracea* var. Botrytis L.): partial purification and properties. Plant Physiol 42: 1763–1768
17. MOORE AL, C JACKSON, B HALLIWELL, JE DENCH, DO HALL 1977 Intramitochondrial localization of glycine decarboxylase in spinach leaves. Biochem Biophys Res Commun 78: 483–491
18. MOTOKAWA Y, G KIKUCHI 1974 Glycine metabolism by rat liver mitochondria. Isolation and some properties of the protein-bound intermediate of the reversible glycine cleavage reaction. Arch Biochem Biophys 164: 634–640
19. MOTOKAWA Y, G KIKUCHI 1974 Glycine metabolism by rat liver mitochondria. Reconstitution of the reversible glycine cleavage system with partially purified protein components. Arch Biochem Biophys 164: 624–633
20. NAKANO Y, M FUJIOKA, H WADA 1968 Studies on serine hydroxymethylase isoenzymes from rat liver. Biochim Biophys Acta 159: 19–26
21. OSMOND CB, B HARRIS 1971 Photorespiration during C_4-photosynthesis. Biochim Biophys Acta 234: 270–282
22. PRATHER CW, CW SISLER 1966 Purification and properties of serine hydroxymethyltransferase from *Nicotiana rustica* L. Plant Cell Physiol 7: 457–464
23. QUEBEDEAUX B, R CHOLLET 1975 Growth and development of soybean (*Glycine max* [L.] Merr.) pods. Plant Physiol 55: 745–748
24. ROBINSON HR, SM KLEIN, RD SAGERS 1973 Glycine metabolism. Lipoic acid as the prosthetic group in the electron transfer protein P_2 from *Peptococcus glycinophilus*. J Biol Chem 248: 5319–5323
25. SAWHNEY SK, MS NAIK, DJD NICHOLAS 1978 Regulation of nitrate reduction by light, ATP and mitochondrial respiration in wheat leaves. Nature 272: 647–648
26. SHAH SPJ, EA COSSINS 1970 The biosynthesis of glycine and serine by isolated chloroplasts. Phytochemistry 9: 1545–1551
27. TAYLOR RH, H WEISSBACH 1965 Radioactive assay for serine transhydroxymethylase. Anal. Biochem 13: 80–84
28. WANG D, RH BURRIS 1963 Carbon metabolism of ^{14}C-labeled amino acids in wheat leaves. II. Serine and its role in glycine metabolism. Plant Physiol 38: 430–439
29. WILKINSON AP, DD DAVIES 1958 Glycine-serine interconversion in plant tissues. Nature 181: 1070–1071
30. WOO KC, JA BERRY, CB OSMOND, GH LORIMER 1977 Photorespiration and the assimilation of ammonia. Abstr 4th Int Congr Photosyn, Reading, U.K. pp 415
31. WOO KC, JA BERRY, G TURNER 1978 Release and refixation of ammonia during photorespiration. Carnegie Inst Wash Year Book '77. In press
32. WOO KC, CB OSMOND 1976 Glycine decarboxylation in mitochondria isolated from spinach leaves. Aust J Plant Physiol 3: 771–785
33. WOO KC, CB OSMOND 1977 Participation of leaf mitochondria in the photorespiratory carbon oxidation cycle: glycine decarboxylation activity in leaf mitochondria from different species and its intramitochondrial location. *In* S Miyachi, S Katoh, Y Fujita, K Shibata, eds, Photosynthetic Organelles. Japan Soc Plant Physiol, Ctr Acad Publ, Tokyo, pp 315–323
34. ZELIKSON R, M LUZZATI 1976 Two forms of serine transhydroxymethylase, one absent in a thymidylate-less mutant in *Saccharomyces cerevisae*. Eur J Biochem 64: 7–13