Phytochemistry, 1975, Vol. 14, pp. 387–391. Pergamon Press. Printed in England.

REGULATION OF MITOCHONDRIAL GLYCINE DECARBOXYLASE FROM PEA MITOCHONDRIA

MICHAEL THOMAS CLANDININ and EDWIN ALBERT COSSINS

Department of Botany, University of Alberta, Edmonton, Canada

(*Received* 4 *February* 1974)

Key Word Index—*Pisum sativum*; Leguminosae; pea; mitochondria; glycinedecarboxylase; C-1 unit synthesis; regulation of methyl group biogenesis.

Abstract—Reactions of glycine cleavage were assayed in mitochondria isolated from cotyledons of germinating pea seeds. These reactions, which included the exchange of bicarbonate with C-1 of glycine and an NAD-stimulated decarboxylation of glycine, were maximal under aerobic conditions at pH 7·8. The apparent Michaelis–Menten constants for glycine and bicarbonate in the exchange reaction were 1·8 and 12·5 mM respectively. The K_m for NAD in the decarboxylation reaction was 47 μM. Maximal enzyme activity was observed when mitochondrial integrity was maintained. Up to 40% inhibition of the decarboxylation reaction was observed when NADH, NADPH or L-methionine were added to the reaction system. When glycine-[2-^{14}C] was incubated with the isolated mitochondria, labelled CO_2 was evolved in nanomolar quantities. It is concluded that glycine decarboxylase may be of importance in supplying C-1 units for the *de novo* synthesis of methionine in pea mitochondria.

INTRODUCTION

Examination of plants has established that glycine decarboxylase activity is localized in the mitochondria of photosynthetic tissue [1, 2]. This finding is compatible with the pathways of glycollate metabolism proposed by Tolbert and co-workers [3–7]. These latter workers have suggested that glycine decarboxylase is responsible for the generation of CO_2 in photorespiration together with the subsequent production of serine from 5,10-CH_2–H_4PteGlu* produced from carbon 2 of glycine upon decarboxylation.

Mitochondria, isolated from germinating pea cotyledons [8], have ability to cleave glycine molecules when glycine-[2-^{14}C] is supplied. The principal labelled products included HCO–H_4PteGlu and 5-CH_3–H_4PteGlu together with serine and methionine. Radioactivity from L-serine-[3-^{14}C] was also incorporated into glycine implying that the splitting reaction may be reversible. Similar incorporations of the α-carbon of glycine into the β-carbon of serine have been clearly established for rat liver [9–16], bacteria [17–19], and other plant species [20]. Extensive study and purification of the enzyme system from *Peptococcus glycinophilus*, which catalyzes the labilization of the carboxyl group of glycine, has also been accomplished [21–24]. In the latter studies detailed observations on the properties of four protein subunits involved in this decarboxylation reaction have been made.

Because the occurrence and physiological significance of the glycine cleavage reaction is not as clearly understood in plant mitochondria, the present work has examined some of the properties of this enzyme with particular emphasis on its possible relationships with the mitochondrial pteroylglutamate pool. The reaction as a potential source of C-1 units for *de novo* synthesis of methionine and related transmethylation reactions has also been considered.

RESULTS

Previous indications that mitochondria may be a site of glycine decarboxylase activity in this tissue [8] are substantiated by the distribution of the glycine–bicarbonate exchange reaction between the soluble and particulate cell fractions (Table 1). Mitochondria, purified by sucrose density gradient ultracentrifugation, exhibited a specific activity for the exchange reaction which was 47-fold greater than that of the unfractionated crude homogenate. Approximately 5% of the total enzyme activity was localized in the mitochondrial fraction (Table 1).

* The abbreviations used for derivatives of pteroylglutamic acid are those suggested by the IUPAC–IUB Commission as listed in *Biochem. J.* (1967) **102**, 15, e.g. 5,10-CH_2-H_4PteGlu = N^5,N^{10}-methylenetetrahydropteroylmonoglutamate.

Table 1. Localization of the glycine–bicarbonate exchange reaction within the soluble and particulate cell fractions

Cell fraction	Total enzyme activity (nmol $^{14}CO_2$/g fresh wt)	Specific enzyme activity (nmol $^{14}CO_2$/mg protein)	Enzyme recovery (% of total)
Crude homogenate	16·4	0·068	100
27000 g particulate fraction	0·82	0·727	5·0
Isolated mitochondria	0·80	3·2	4·8

The complete reaction mixtures (2 ml) contained: 1·3 mmol sucrose, 0·5 μCi glycine-[1-^{14}C] (0·05 μCi/μmol), 60 μmol $NaHCO_3$, 100 μmol Tris–HCl buffer (pH 7·8), 1·5 μmol pyridoxal-5′-phosphate, 10 μmol dithiothreitol, and the cell fraction (2 mg protein). Reaction mixtures were incubated aerobically for 30 min at 37°.

Instability of this enzyme as a potentially important factor affecting the specific activities recovered, can be ruled out, as loss of activity during a 24 hr storage period was minimal.

The requirements for optimum exchange of bicarbonate with glycine are shown in Table 2 and included bicarbonate, sulphydryl groups, pyridoxal phosphate and mitochondrial protein. Addition of H_4PteGlu reduced [^{14}C]CO_2 recovered in the exchange reaction. Sucrose (0·65 M) was present in all reaction systems as an osmoticum because preliminary assays without this compound gave very low glycine decarboxylase or glycine–bicarbonate exchange activities. In further experiments disruption of mitochondrial integrity by solubilizing treatments such as sonication, triton X100, digitonin and deoxycholate reduced the amount of glycine–bicarbonate exchange by as much as 60%. The exchange reaction occurred under aerobic and anaerobic conditions at approximately equal rates. In the presence of dithiothreitol enzyme activity in air was slightly higher than in pure N_2. Under optimum conditions of assay the rate of glycine–bicarbonate exchange was linear for at least 1 hr and in Tris–HCl buffer showed a pH optimum of 7·8. Product formation increased linearly as mitochondrial protein concentration was increased to 10 mg/reaction system, and a similar linear relationship was found for bicarbonate concentrations up to 18 mM. Enzyme activity was also affected by glycine concentration (Fig. 1) and Lineweaver–Burk plots [25] for this substrate and for bicarbonate gave apparent K_m values of 1·8 mM and 12·5 mM respectively. These values are similar in magnitude to those reported for the enzyme of rat liver [10, 11]. The glycine cleavage or decarboxylase reaction was stimulated by NAD^+ in the absence of bicarbonate (Fig. 2a) with an apparent K_m of 47 μM. The cleavage reaction was also partially inhibited by reduced pyridine nucleotides (Fig. 2b).

Several properties of the glycine cleavage and exchange reactions appear to suggest requirement for an oxidation–reduction balance that could be maintained by a structural component of the mitochondrial membrane. In this regard, Bird *et al.* [26] have reported that conversion of two glycine molecules to serine by particulate preparations of tobacco leaves required O_2, was inhi-

Table 2. Requirements of the glycine bicarbonate exchange reaction

Omission from the reaction system	Enzyme activity (nmol $^{14}CO_2$/mg protein)
None	2·8
Mitrochondrial fraction	n.d.*
Dithiothreitol	2·2
Pyridoxal-5′-phosphate	1·2
Bicarbonate	0·6
None plus 1 μmol NAD	1·6
None plus 0·2 μmol H_4PteGlu	2·2

* n.d. = Not detected.

The complete reaction mixture (see Table 1) was incubated for 30 min at 37° after each reaction flask was flushed with N_2.

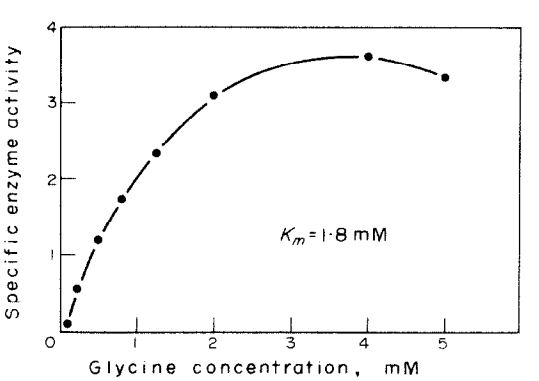


Fig. 1. The effect of glycine concentration on the rates of the exchange reaction. The complete reaction mixture was as in Table 1.

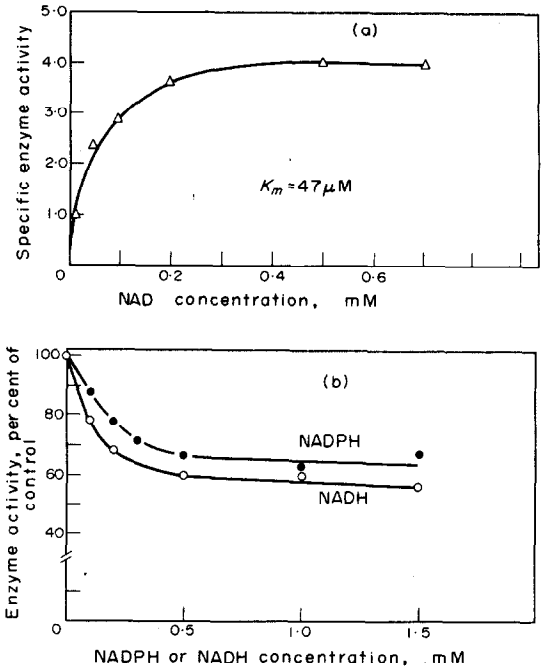


Fig. 2. The effect of pyridine nucleotides on glycine decarboxylase activity. The concentration of NAD (a), NADPH (b) was varied as illustrated. Other components of the reaction system are described in the experimental section. The control reaction system produced 3·5 nmol of $[^{14}C]CO_2$ per mg of protein per 30 min.

bited by inhibitors of mitochondrial electron transport and was stimulated by ADP. It was concluded that ATP was synthesized at the expense of free energy derived from glycine cleavage. In mitochondria isolated from pea cotyledons, however, ADP did not stimulate glycine cleavage (Fig. 3). The cleavage reaction was affected by various intermediates or products of mitochondrial C-1 metabolism. In this respect, methionine and 5-Me-H_4PteGlu were effective in reducing glycine decarboxylase activity whereas *S*-adenosyl-L-methionine (SAM) was not (Fig. 3).

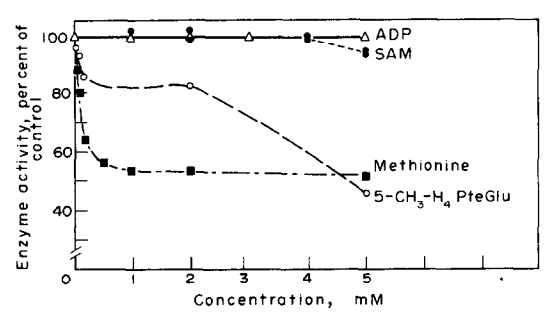


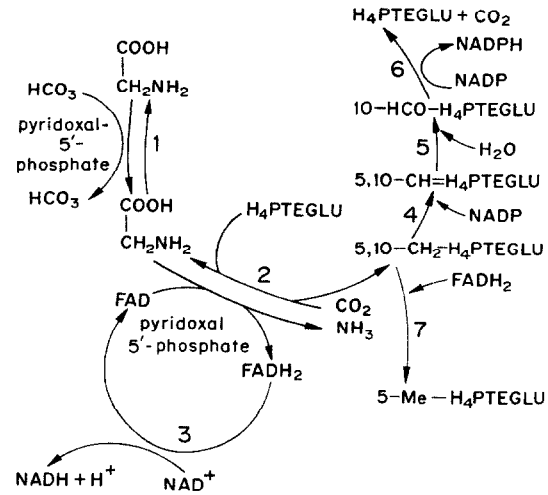
Fig. 3. The effect of L-methionine, 5-Me-H_4PteGlu, SAM and ADP on glycine decarboxylase activity. Supplements to the complete reaction system outlined in the experimental section were added in the concentrations illustrated.

The cleavage of glycine by some animal tissues is a significant catabolic process resulting in complete oxidation of the molecule to CO_2. Because such oxidation would necessitate some involvement of the pteroylglutamate pool, a study was undertaken to determine if glycine-[2-^{14}C] would generate $^{14}CO_2$ when incubated with the isolated mitochondria. When glycine-[2-^{14}C] (specific activity 21·8 μCi/μmol) was incubated with isolated mitochondria for 30 min at 37° in air, approximately 0·02 nmol of $[^{14}C]CO_2$ was collected. This amount was not increased by additions of NADP or H_4PteGlu.

DISCUSSION

The results of the present study indicate that isolated pea mitochondria can cleave glycine molecules. In many respects the properties of this plant system appear to parallel those of systems isolated from mammalian liver [9–16] and *Peptococcus glycinophilus* [21–24]. For example, pea mitochondria readily catalyzed the exchange of bicarbonate with the carboxyl carbon of glycine (Table 2) as well as an NAD-dependent decarboxylation of glycine (Fig. 2). Further similarities are noted in the apparent K_m values shown for bicarbonate, glycine and NAD. On this basis, it is conceivable that plant mitochondria may metabolize glycine by the pathway proposed for rat liver [9–16] and *Peptococcus glycinophilus* [21–24]. Degradation of glycine molecules by this route (Scheme 1) would generate CO_2, NH_3 and 5,10-CH_2-H_4PteGlu. Production of the latter compound from carbon-2 of glycine would be logically followed by its ulitization in mitochondrial pteroylglutamate metabolism for a variety of reactions involving hydroxymethylation, formylation, and methylation [8, 27]. Evidence for these latter reactions, which involve oxidation and reduction of the C-1 unit, has already been obtained from our earlier studies [8].

The reversible exchange of carbon-1 of glycine with bicarbonate (Scheme 1, reaction 1) appeared to require only pyridoxal-5′-phosphate and intact mitochondria for optimum activity (Table 2). Addition of NAD or H_4PteGlu resulted in some inhibition of the exchange reaction (Table 2). On the other hand, the decarboxylation of glycine



Scheme 1. Glycine and serine catabolism in pea mitochondria.

(Scheme 1, reaction 2) had requirements for NAD, and pyridoxal-5′-phosphate (Fig. 2a). The inhibition observed by reduced pyridine nucleotides (Fig. 2b) suggests that a particular oxidation–reduction balance might be necessary to maintain decarboxylation. A direct requirement for H_4PteGlu in the cleavage reaction was not observed in the present work. However, as the mitochondria have ability to generate H_4PteGlu from endogenous folate derivatives [8], sufficient levels of H_4PteGlu were probably present in the reaction system to satisfy the requirements of Scheme 1, reaction 2.

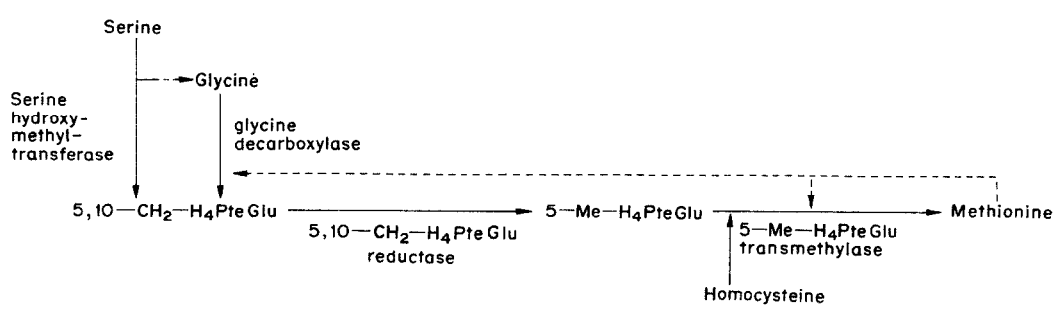
The occurrence in pea mitochondria of reactions which lead to CO_2 production from C-2 of glycine (Scheme 1, reactions 4, 5, 6) is indicated by the observation that some $^{14}CO_2$ was generated when glycine-[2-^{14}C] was supplied. This may not, however, represent a major pathway for mitochondrial glycine metabolism as our previous studies [8, 27] show that C-1 units arising within this organelle are utilized in the synthesis of methionine (Scheme 1, reaction 7). In this regard it may be of significance that L-methionine affects the activities of the mitochondrial glycine decarboxylase (Fig. 3) and 5-Me-H_4PteGlu:homocysteine methyltransferase [8]. The inhibition of glycine decarboxylase by 5-Me-H_4PteGlu (Fig. 3) is probably caused by methionine formed by the mitochondrial 5-Me-H_4PteGlu methyltransferase. This end product-regulated synthesis of methionine may be facilitated by a similarly controlled generation of C-1 units from carbon 2 of glycine (Scheme 2) and carbons 2 and 3 of serine (Scheme 2). The interrelationships and nature of the control mechanisms which regulate glycine and serine metabolism in this plant organelle, however, remain to be thoroughly elucidated.

EXPERIMENTAL

Materials. Glycine-[2-^{14}C] and glycine-[1-^{14}C] were purchased from Amersham Searle Corp., Des Plaines, Ill., U.S.A. Other chemicals were purchased from Fisher Scientific Supplies Ltd., Edmonton, Alberta, Canada or Sigma Chemical Co., St. Louis, Mo., U.S.A. Seeds of *Pisum sativum* L. cv. Homesteader were germinated for 96 hr at 25° as previously described [8].

Preparation of particulate fraction. All operations were carried out at 2–4°. Mitochondria were isolated from 4-day-old cotyledon tissue and purified on a sucrose density gradient as previously described [8]. The mitochondrial fraction isolated was used as a source of particulate enzyme for assays of glycine decarboxylase or glycine–bicarbonate exchange reactions.

Enzyme assays. The exchange of bicarbonate with the carboxyl carbon of glycine-[1-^{14}C] was assayed using a reaction system similar to that of Sato *et al.* [10], with the following modifications. Reactions were carried out aerobically (20% O_2) except in experiments where the effects of O_2 tensions were examined. The reaction components, in stoppered Warburg manometric flasks, were incubated at 37° for 30 min. The complete reaction system in a total vol. of 2 ml contained: 10 μmol glycine-[1-^{14}C] (0·05 μCi/μmol), 1·5 μmol pyridoxal-5′-phosphate, 10 μmol dithiothreitol, 100 μmol Tris–HCl buffer (pH 7·8 at 37°), 60 μmol $NaHCO_3$, 1·3 mM of sucrose and aliquots of the mitochondrial suspension containing 2 mg of protein. The



Scheme 2. Feedback inhibition of glycine decarboxylase and 5-CH_3-H_4PteGlu transmethylase by methionine.

reaction was stopped, and the $^{14}CO_2$ liberated simultaneously from the solution by addition of 0·3 ml of 4 N H_2SO_4 tipped from the side arm of the flask. The CO_2 liberated was absorbed in 0·4 ml of 50% (v/v) hyamine hydroxide in MeOH previously placed in the centre well of the flask. The flasks were agitated at 50° for 45 min to complete CO_2 absorption. The contents of the centre well, in 15 ml of Bray's solution [28], were counted by liquid scintillation spectrometry as previously described [8].

Glycine decarboxylase assays were carried out under the same conditions, except 1·25 μmol NAD^+ and 0·2 μmol H_4PteGlu were added, while $NaHCO_3$ was omitted. Further variations in these two reaction systems and their controls are specifically outlined in the respective tables or figures. Sp. act. is expressed as nmol $^{14}CO_2$ liberated/mg protein in 30 min at 37°. Protein was measured by a colorimetric method utilizing crystalline egg albumin as a reference standard [29].

Solubilization treatments. Several solubilization treatments were performed to determine the effect of disrupting mitochondrial integrity on enzyme activity. The solubilization treatments were carried out for 2 min at 0° using the following solubilizer: Triton X100 (0·1% v/v), digitonin (0·1% w/v) or deoxycholate (0·1% w/v). The effect of mechanical disruption was also tested by sonicating the isolated mitochondria with a Fisher Ultrasonic Generator, Model BPO (Blackstone Ultrasonics Inc., Sheffield, Pa., U.S.A.) at full amplification for 1 min at 4°.

Acknowledgements—This work was supported by a grant-in-aid of research awarded to one of us (E.A.C.) by the National Research Council of Canada. The senior author was the recipient of a National Research Council of Canada Post-Graduate Scholarship during this investigation.

REFERENCES

1. Kisaki, T., Imai, A. and Tolbert, N. E. (1971) *Plant and Cell Physiol.* **12,** 267.
2. Kisaki, T., Yoshida, N. and Imai, A. (1971) *Plant and Cell Physiol.* **12,** 275.
3. Tolbert, N. E. (1962) *Nat. Acad. Sci. Nat. Res. Council Publ.* No. 1145.
4. Tolbert, N. E., Oeser, A., Kisaki, T., Hageman, R. H. and Yamazaki, R. K. (1968) *J. Biol. Chem.* **243,** 5179.
5. Tolbert, N. E., Oeser, A., Yamazaki, R. K., Hageman, R. H. and Kisaki, T. (1969) *Plant Physiol.* **44,** 135.
6. Kisaki, T. and Tolbert, N. E. (1969) *Plant Physiol.* **44,** 242.
7. Bruin, W. J., Nelson, E. B. and Tolbert, N. E. (1970) *Plant Physiol.* **46,** 386.
8. Clandinin, M. T. and Cossins, E. A. (1972) *Biochem. J.* **128,** 29.
9. Kawasaki, H., Sato, T. and Kikuchi, G. (1966) *Biochem. Biophys. Res. Commun.* **23,** 227.
10. Sato, T., Kochi, H., Motokawa, Y., Kawasaki, H. and Kikuchi, G. (1969) *J. Biochem.* **65,** 63.
11. Sato, T., Kochi, H., Sato, N. and Kikuchi, G. (1969) *J. Biochem.* **65,** 77.
12. Motokawa, Y. and Kikuchi, G. (1969) *Arch. Biochem. Biophys.* **135,** 402.
13. Motokawa, Y. and Kikuchi, G. (1969) *J. Biochem.* **65,** 71.
14. Motokawa, Y. and Kikuchi, G. (1971) *Arch. Biochem. Biophys.* **146,** 461.
15. Yoshida, T. and Kikuchi, G. (1970) *Arch. Biochem. Biophys.* **139,** 380.
16. Yoshida, T. and Kikuchi, G. (1971) *Arch. Biochem. Biophys.* **145,** 658.
17. Nash, T. (1953) *Biochem. J.* **55,** 416.
18. Osborn, M. J., Talbert, P. T. and Heunnekens, F. M. (1960) *J. Am. Chem. Soc.* **82,** 4921.
19. Van Slyke, D. D., Dillon, R. T., McFadyen, D. A. and Hamilton, P. (1941) *J. Biol. Chem.* **141,** 627.
20. Prather, C. W. and Sisler, E. C. (1972) *Phytochemistry* **11,** 1637.
21. Klein, S. M. and Sagers, R. D. (1966a) *J. Biol. Chem.* **241,** 197.
22. Klein, S. M. and Sagers, R. D. (1966b) *J. Biol. Chem.* **241,** 206.
23. Klein, S. M. and Sagers, R. D. (1967a) *J. Biol. Chem.* **242,** 297.
24. Klein, S. M. and Sagers, R. D. (1967b) *J. Biol. Chem.* **242,** 301.
25. Lineweaver, H. and Burk, D. (1934) *J. Am. Chem. Soc.* **56,** 658.
26. Bird, I. F., Cornelius, M. J., Keys, A. J. and Whittingham, C. P. (1972) *Phytochemistry* **11,** 1587.
27. Clandinin, M. T. and Cossins, E. A. (1974) *Phytochemistry* **13,** 585.
28. Bray, G. A. (1960) *Anal. Biochem.* **1,** 279.
29. Lowry, O. H., Rosebrough, N. J., Farr, A. J. and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265.