

Oxidative Decarboxylation of Glycollate and Glyoxylate by Leaf Peroxisomes

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1. The conditions under which peroxisomal preparations from leaves of spinach beet and spinach catalyse the release of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glycollate and $[1-^{14}\text{C}]$ glyoxylate were investigated. 2. At pH 8, $^{14}\text{CO}_2$ production from $[1-^{14}\text{C}]$ glyoxylate was accompanied by equivalent quantities of formate. The accumulation of oxalate and the effects of various reagents, especially catalase inhibitors, show that glyoxylate is non-enzymically oxidized by H_2O_2 , which is generated by the oxidation of glyoxylate to oxalate by the action of glycollate oxidase. 3. $^{14}\text{CO}_2$ is shown to be generated from $[1-^{14}\text{C}]$ glycollate at pH 8 by a similar reaction, but the H_2O_2 is generated mainly by the oxidation of glycollate to glyoxylate. 4. The physiological significance of these reactions is discussed, with special reference to their role in photorespiration.

Illuminated green leaves release fixed CO_2 in a light-dependent oxidation process known as photorespiration (Jackson & Volk, 1970). This CO_2 arises mainly from the carboxyl group of glycollate; little is released from the α -carbon atom (Zelitch, 1966). Two series of reactions have been proposed to account for this decarboxylation of glycollate. In the first, glycollate is oxidized to glyoxylate by the action of glycollate oxidase, the glyoxylate is converted into glycine by aminotransferases and this glycine then decarboxylated by an enzyme system located in the mitochondria (Tolbert, 1971; Kisaki *et al.*, 1971*a,b*; Prather & Sisler, 1972). In the second, the glyoxylate generated by glycollate oxidation is oxidized to formate and CO_2 by illuminated chloroplasts (Zelitch, 1972). Since both glycollate oxidase and the aminotransferases are located in peroxisomes (Tolbert, 1971; Rehfeld & Tolbert, 1972), the generation of CO_2 from glycollate would require the movement of intermediates from peroxisomes to mitochondria or chloroplasts respectively. The release of CO_2 from glycollate and glyoxylate by reactions taking place entirely within peroxisomes is described here and the contribution of these reactions to photorespiration discussed.

A preliminary account of this work has been presented (Halliwell *et al.*, 1972).

Materials and Methods

Materials

Chemicals. Reagents were of the highest purity available from Sigma Chemical Co., Kingston-upon-Thames, Surrey, U.K., or from BDH Chemicals Ltd.,

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Poole, Dorset, U.K. $[1-^{14}\text{C}]$ Glycine and the sodium salts of $[1-^{14}\text{C}]$ glycollate, $[1-^{14}\text{C}]$ glyoxylate and $[2-^{14}\text{C}]$ glyoxylate were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. $[2-^{14}\text{C}]$ -Glycollate (calcium salt) was from I.C.N. Corp., Calif., U.S.A. Solutions of radioactive glycollate and glyoxylate were stored at -20°C until required; chromatographic analysis of these solutions showed that no decomposition took place over a 1-week period, the maximum time for which any solution was stored.

Butyl-PBD [5-(4-biphenyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole] for liquid-scintillation radioactivity counting was obtained from CIBA-Geigy Ltd., Duxford, Cambridge, U.K.

Plants. Spinach-beet leaves were harvested from plants grown outdoors in the summer and, in the winter, grown in the greenhouse (receiving supplementary mercury lighting for 4h each day), or were purchased locally. No difference in the results with preparations from these different sources was observed. Spinach leaves were purchased locally. Only undamaged adult leaves were used.

Methods

Preparation of subcellular fractions. Homogenates of leaves of spinach beet were fractionated by the method of Leek *et al.* (1972). The homogenate was first centrifuged at 500g and the supernatant from this centrifuged at 6000g. The precipitate sedimented at 6000g was resuspended in homogenizing medium and layered above 1.8M-sucrose in 20mM-glycylglycine-KOH buffer, pH 7.5. After centrifuging at 76000*g*_{av.} for 3.5h, the peroxisome fraction formed a pellet on the bottom of the tube. This pellet was resuspended in homogenizing medium.

Homogenates of spinach leaves were subjected to differential centrifugation, followed by analysis of the resuspended 6000g precipitate on a sucrose gradient, as described by Tolbert *et al.* (1969). Peroxisomal and mitochondrial fractions were withdrawn from the gradient by piercing the bottom of the tube and used directly in assays.

Assay of decarboxylation reactions. The release of $^{14}\text{CO}_2$ from ^{14}C -labelled substrates was measured. The reaction mixtures were incubated with shaking at 25°C , usually for 2h, in tightly stoppered 25ml flasks. Each flask was fitted with a small plastic tube containing 0.2ml of 20% (w/v) KOH to collect the $^{14}\text{CO}_2$. When the pH of the reaction mixture was 5 or below, the tube was removed after the appropriate time and 0.1ml of its contents added to 5ml of scintillation fluid (composition: 7g of butyl-PBD and 80g of naphthalene dissolved in 600ml of toluene and 400ml of 2-methoxyethanol), which was counted for radioactivity in a Tracerlab Corumatic/200 liquid-scintillation counter. The amount of isotope present in the reaction mixture was counted for radioactivity under the same conditions and gave about 5×10^5 c.p.m. for every $0.25\mu\text{Ci}$ added. When the pH of the reaction mixture was above 5, 0.2ml of 2.5M- H_2SO_4 was added to the reaction mixture after the appropriate time and incubation at 25°C was continued for a further 60min before sampling the KOH. Addition of acid at the beginning of the assays completely abolished release of $^{14}\text{CO}_2$.

All assays were accompanied by controls in which extract was omitted, or by using extract that had been heated at 100°C for 10min. Unless otherwise stated, the rates of non-enzymic decarboxylation measured in this way were less than 10% of the enzymic rates, and the results presented have been corrected for these small non-enzymic rates. In all of the assays listed below, the net rate of $^{14}\text{CO}_2$ release from all the substrates was proportional to the amount of extract added up to at least twice that normally used, and was linear with time for at least 2.5h, unless otherwise stated.

Standard assays. The standard assays used were as follows.

(a) Glyoxylate. Release of $^{14}\text{CO}_2$ from [^{14}C]glyoxylate was assayed by incubating 5mM-sodium glyoxylate, containing $0.25\mu\text{Ci}$ of either [^{14}C]glyoxylate or [^{14}C]glyoxylate, with 0.03mM-FMN, 0.2ml of extract and 33mM-glycylglycine buffer, adjusted to pH8 with KOH, in a total volume of 3ml for 2h at 25°C .

(b) Glycollate. Release of $^{14}\text{CO}_2$ from [^{14}C]glycollate was assayed by incubating 5mM-sodium glycollate, containing $0.25\mu\text{Ci}$ of [^{14}C]glycollate, with 0.03mM-FMN, 0.1ml of extract and 33mM-glycylglycine buffer, adjusted to pH8 with KOH, in a total volume of 3ml for 2h at 25°C . For measuring the release of $^{14}\text{CO}_2$ from [^{14}C]glycollate, $0.5\mu\text{Ci}$ of

isotope, 0.2ml of extract, 0.03mM-FMN and 33mM- K_2HPO_4 buffer, adjusted to pH5 with citric acid, were incubated under the same conditions.

Protecting the reaction mixtures from light by wrapping the flasks in aluminium foil did not affect the properties of any of these reactions. The inclusion of 0.01% Triton X-100 in the reaction mixtures did not increase $^{14}\text{CO}_2$ release from any of these substrates.

Determinations. Formate was measured by reduction to formaldehyde, which forms a yellow complex with acetylacetone, measured spectrophotometrically at 412nm (Wood & Gest, 1957). Chlorophyll was determined by the method of Arnon (1949). Protein was precipitated from solution with 5% (w/v) trichloroacetic acid, dissolved in 0.1ml of 3% (w/v) NaOH and assayed by the Lowry method as described by Leggett-Bailey (1962), with crystalline bovine serum albumin, desiccated before use, as a standard.

Chromatography. Glyoxylate, oxalate and glycollate were separated by descending chromatography on Whatman no. 1 paper in 2-methoxyethanol-pyridine-acetic acid-water (8:4:4:1, by vol.). All solvents contained 0.15% (w/v) 8-hydroxyquinoline. Spots containing glyoxylate were applied to the paper immediately before developing the chromatogram, as drying can cause decomposition of this compound. After 10h, the paper was dried thoroughly and cut into strips (1cm \times 3cm) which were placed in vials containing 5ml of scintillation fluid (composition: 5g of butyl-PBD/litre of toluene) and counted for radioactivity in the Tracerlab scintillation counter. Appropriate standards were run with each chromatogram; oxalate was found close to the origin, whereas the R_F values of glycollate and glyoxylate were 0.58 and 0.46 respectively.

Results

Release of CO_2 from glyoxylate

Effect of pH. Peroxisomal preparations obtained from leaves of spinach beet readily catalysed the decarboxylation of glyoxylate. Fig. 1(a) shows the effect of pH on the rate of this reaction. Two peaks of activity were observed, with optima at pH4-5 and 8-8.5 respectively. In contrast, the rate of release of $^{14}\text{CO}_2$ from [^{14}C]glyoxylate was significant only below pH6 (Fig. 1b). At pH5, the rates of $^{14}\text{CO}_2$ release from [^{14}C]glyoxylate were one-third to one-half of those from [^{14}C]glyoxylate whereas, at pH8, CO_2 was released only from the carboxyl group. Since CO_2 release during photorespiration arises only from the carboxyl group of glycollate, this decarboxylation of glyoxylate at pH8 might play some part in photorespiration and was further investigated.

Assays at pH8 were usually carried out with glycylglycine-KOH buffer. Although higher rates

were obtained with potassium phosphate-citrate buffer (Fig. 1a), its poor buffer capacity at pH8 precluded its routine use. Tris could not be used, as it forms a complex with glyoxylate (Thompson & Richardson, 1968), and Good's buffers (Good *et al.*, 1966) caused a rapid non-enzymic decarboxylation of glyoxylate at pH8 (Halliwell & Butt, 1972). Although 20mM-glyoxylate was required to saturate the system (Table 1), 5mM-glyoxylate was usually used, without any apparent effect on the reaction characteristics, because of the cost of glyoxylate. In a series of 13 peroxisomal preparations, the rate of glyoxylate decarboxylation at pH8 was 1.13 ± 0.093 (mean \pm s.d.) $\mu\text{mol/h}$ per mg of protein, but higher rates could be obtained at higher substrate concentrations or in the presence of phosphate-citrate buffer.

Subcellular distribution. A crude homogenate of spinach-beet leaves was subjected to differential centrifugation, followed by centrifugation of the resuspended 6000g precipitate through a column of 1.8M-sucrose (see the Materials and Methods section). Fig. 2 shows the distribution of glyoxylate decarboxylation activity at pH8, together with that of various marker enzymes. The activity was mainly non-particulate on differential centrifugation, but 30% of the activity of the 6000g precipitate applied to the sucrose column was found in the pellet. This distribution very closely resembled that of glycollate oxidase, a peroxisomal marker enzyme used by Tolbert *et al.* (1969), but not that of chlorophyll nor of cytochrome oxidase. The sucrose column removed all of the chlorophyll and most of the mitochondria from the material applied, giving a pellet with a specific activity for glyoxylate decarboxylation much higher than that of the initial homogenate. Assuming that glycollate oxidase is released from the peroxisomes during homogenization and fractionation, it is concluded that the glyoxylate oxidation activity is also associated with peroxisomes.

Table 1. Effect of substrate concentration on rate of glyoxylate decarboxylation by spinach-beet peroxisomes

The peroxisomal preparation was incubated with glyoxylate, containing $[1-^{14}\text{C}]$ glyoxylate, as described in the Materials and Methods section.

Concn. of glyoxylate (mM)	Rate of decarboxylation (μmol of CO_2 released/h per ml of extract)
1	0.082
2	0.248
5	0.953
10	2.071
20	2.818
40	2.857

Effect of metabolites and inhibitors. The effect of various reagents on glyoxylate decarboxylation by peroxisomal preparations was investigated. The results are presented in Table 2. Among likely intermediates, neither formate (Leek *et al.*, 1972) nor glycine (Kisaki & Tolbert, 1970) were involved in the release of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glyoxylate at pH8, as addition of these compounds did not decrease the $^{14}\text{CO}_2$ released. Further, $^{14}\text{CO}_2$ release from $[1-^{14}\text{C}]$ -glycine was not detected under these conditions.

Mitochondrial systems for glyoxylate decarboxylation have been reported; these require thiamin pyrophosphate and divalent metal ions (Davies & Corbett, 1969; Prather & Sisler, 1972). However, addition of these cofactors had little effect on glyoxylate decarboxylation by peroxisomes. The addition of EDTA, which would chelate any metal ions that might be present in the extract, had no effect. Glyoxylate decarboxylation by peroxisomes seems therefore to be unrelated to the reaction catalysed by mitochondria.

The most striking effects were obtained with catalase inhibitors. 3-Amino-1,2,4-triazole (Margoliash & Novogradsky, 1958), azide or β -mercaptoethanol all markedly increased the rate of $^{14}\text{CO}_2$

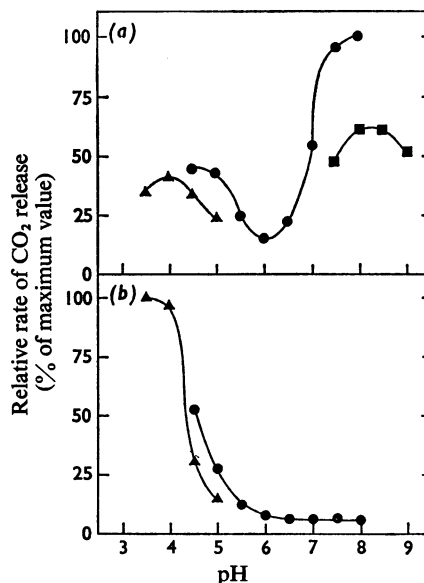


Fig. 1. Effect of pH on release of $^{14}\text{CO}_2$ from (a) $[1-^{14}\text{C}]$ -glyoxylate and (b) $[2-^{14}\text{C}]$ -glyoxylate

The reaction conditions were those described in the Materials and Methods section: 5mM-glyoxylate, containing $0.25 \mu\text{Ci}$ of either $[1-^{14}\text{C}]$ glyoxylate or $[2-^{14}\text{C}]$ -glyoxylate, was incubated with 0.2ml of a spinach-beet peroxisomal preparation in 33mM-acetic acid-acetate buffer (\blacktriangle); 33mM- K_2HPO_4 -citric acid buffer (\bullet); or in 33mM-glycylglycine-KOH buffer (\blacksquare).

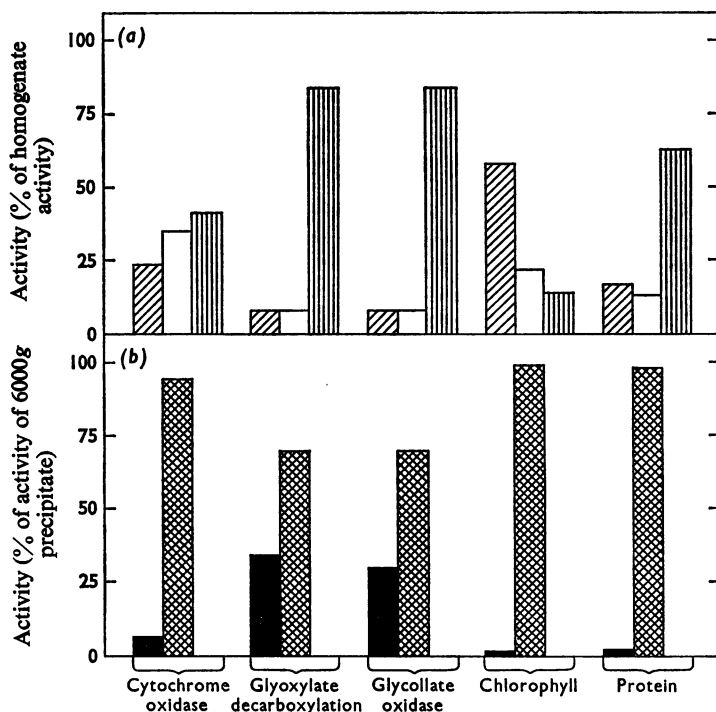
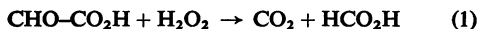


Fig. 2. Subcellular location of glyoxylate decarboxylation activity in spinach-beet homogenates

Fractionation and assay of glyoxylate decarboxylation were carried out as described in the Materials and Methods section. Cytochrome oxidase was assayed by the method of Tolbert *et al.* (1969), glycollate oxidase by the method of Feierabend & Beevers (1972). The height of each bar gives the percentage of total activity recovered in the fraction; the activities recovered varied from 93 to 105% of the homogenate activity and 6000g precipitate activity respectively. (a) Recoveries after differential centrifugation: 500g precipitate (oblique lines ■); 6000g supernatant (unshaded section, □); 6000g supernatant (vertical lines, ▨). (b) Recoveries after sedimenting 6000g precipitate through 1.8M-sucrose: pellet (shaded area, ■); supernatant (cross-hatched area, ▨).

release. The addition of catalase markedly decreased the reaction but did not eliminate it. These results strongly suggest that H_2O_2 participates in the decarboxylation. Glyoxylate is oxidized non-enzymically by H_2O_2 to formate and CO_2 at pH 8 (Hatcher & Holden, 1925; Davies & Corbett, 1969; Halliwell & Butt, 1972) and this reaction may well be involved in the glyoxylate decarboxylation reported here (reaction 1):



In support of this, the production of formate was demonstrated (Table 3). The amounts of CO_2 released and formate produced were almost equal, as might be expected from the stoichiometry of reaction (1). When the amount of $^{14}CO_2$ released was increased by the addition of azide, the amount of formate produced increased correspondingly. It seems likely that glyoxylate decarboxylation is due to production of H_2O_2 by some enzymic reaction (since

activity is abolished by heating) before the H_2O_2 reacts non-enzymically with glyoxylate.

When the reaction vessels were evacuated and filled with argon, the rate of CO_2 release was decreased to less than 20% of that in air. Since the non-enzymic glyoxylate decarboxylation by added H_2O_2 does not require O_2 , it follows that H_2O_2 generation by the preparation is an O_2 -requiring process.

The marked inhibition of glyoxylate decarboxylation by pyrid-2-yl- α -hydroxymethanesulphonate suggests that glycollate oxidase, inhibited by this reagent (Zelitch, 1971), is involved. Both glycollate and FMN stimulated the rate of the reaction markedly (Table 2), and 0.03 mM-FMN was usually included in the reaction mixture. At pH 8, H_2O_2 can be generated by the oxidation of glyoxylate to oxalate, catalysed by glycollate oxidase (reaction 2):

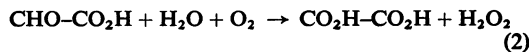


Table 2. *Effect of various reagents on glyoxylate decarboxylation at pH8 by spinach-beet peroxisomes*

Details of incubation are given in the Materials and Methods section. The values in parentheses indicate the rate with reagent added as a percentage of the corresponding control rate.

Reagent	Rate of glyoxylate decarboxylation (μmol of CO_2 released/h per mg of protein)	
	Without reagent	Reagent present
1 mM-Glycine	1.23	1.23 (100)
1 mM-Oxalate	1.00	0.58 (58)
1 mM-Formate	1.00	1.00 (100)
1 mM-Glycollate	1.00	3.12 (312)
0.03 mM-FMN*	1.47	2.19 (149)
0.5 mM-FMN*	1.47	2.18 (148)
0.2 mM-Thiamin pyrophosphate + 1 mM-MgCl ₂	1.00	1.10 (110)
1 mM-EDTA	1.00	1.00 (100)
0.7 mM- β -Mercaptoethanol	1.23	1.87 (152)
17 mM-3-Amino-1,2,4-triazole	1.00	2.88 (288)
1 mM-Azide	1.00	2.81 (281)
1 mM-Pyrid-2-yl- α -hydroxymethanesulphonate	1.00	0.27 (27)
1800 units of ox catalase†	1.47	0.18 (12)

* Except for these two experiments, all flasks contained 0.03 mM-FMN.

† One unit of catalase catalysed the decomposition of $1 \mu\text{mol}$ of H_2O_2 /min when assayed as described by Luck (1963).

The reaction has been shown to be competitively inhibited by oxalate (Richardson & Tolbert, 1961; Tolbert *et al.*, 1949), and the enzyme has a K_m of 5.4 mM for glyoxylate. The inhibition of glyoxylate decarboxylation by added oxalate and the high substrate concentrations required to saturate this system provide further evidence that reaction (2) is the source of H_2O_2 .

This was directly confirmed by chromatographic analysis of reaction mixtures. In a typical experiment, after correction for a control by using a heated preparation, 242 nmol of CO_2 had been released after 2h. However, 963 nmol of oxalate had accumulated in the reaction mixture. Thus more oxalate, and hence H_2O_2 , had been produced than CO_2 released. Much of this H_2O_2 produced would be destroyed by the catalase present in the peroxisomes before it could react with glyoxylate, and this accounts for the observed stimulation of glyoxylate decarboxylation by catalase inhibitors (Table 2). The oxalate produced was not further oxidized at pH8 (Leek *et al.*, 1972).

Table 3. *Production of formate from glyoxylate by spinach-beet peroxisomal preparations*

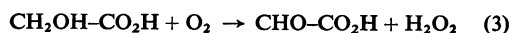
Duplicate flasks and controls were incubated with [$1\text{-}^{14}\text{C}$]glyoxylate as described in the Materials and Methods section. After 2h the formate content of one flask was immediately determined; 0.2 ml of 2.5 M- H_2SO_4 was added to the other flask, which was then incubated for a further 60 min to expel the $^{14}\text{CO}_2$ retained in the reaction mixture, before sampling the KOH. Sodium azide (1 mM) was added to the reaction mixtures where indicated. Expts. A and B refer to experiments with different peroxisome preparations.

Expt.	Reagent added	CO_2 released (μmol)	Formate produced (μmol)
A	None	0.47	0.44
B	None	0.38	0.36
B	1 mM-Azide	2.27	2.11

Release of CO_2 from glycollate

Spinach-beet peroxisomal preparations also catalysed the release of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]glycollate. The pH optimum was 8, and little activity was found below pH6 (Fig. 3a). The rate of decarboxylation, assayed at 5 mM-glycollate, sufficient to saturate the system, was about $1.53 \mu\text{mol/h}$ per mg of protein. In contrast, these preparations catalysed only a very slow release of $^{14}\text{CO}_2$ from [$2\text{-}^{14}\text{C}$]glycollate (Fig. 3b); the average rate for these preparations was $0.04 \mu\text{mol/h}$ per mg of protein at pH5.

Glycollate decarboxylation at pH8 was not linear with time, but followed an accelerating curve (Fig. 4). Nevertheless, the amount of CO_2 released in 2h was directly proportional to the volume of extract added up to three times that normally used. Table 4 shows the effect of metabolites and inhibitors on glycollate decarboxylation. $^{14}\text{CO}_2$ release was decreased by the presence of unlabelled glyoxylate, indicating that glyoxylate is an intermediate in CO_2 release from glycollate. The involvement of glycollate oxidase is also indicated by the stimulation when FMN, which was otherwise added as a routine to reaction mixtures, was included and complete inhibition by pyrid-2-yl- α -hydroxymethane sulphonate. EDTA, glycine and formate had little effect on glycollate decarboxylation. The catalase inhibitors, azide and 3-amino-1,2,4-triazole, stimulated the rate of glycollate decarboxylation markedly. It therefore seems likely that the oxidation of glycollate to glyoxylate by glycollate oxidase (reaction 3) generates H_2O_2 :



Some H_2O_2 then reacts non-enzymically with this glyoxylate. This was directly confirmed by chromatographic analysis of the reaction mixture.

No glycollate was metabolized in control experiments, for which the preparations had been heated

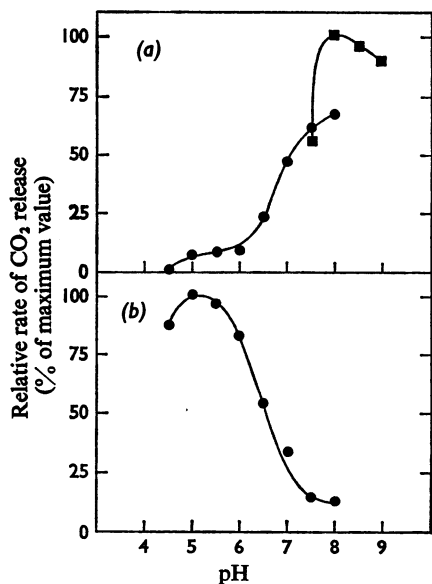


Fig. 3. Effect of pH on the release of $^{14}\text{CO}_2$ from (a) $[1\text{-}^{14}\text{C}]$ glycollate and (b) $[2\text{-}^{14}\text{C}]$ glycollate

Glycollate (5 mM), containing either $0.25\ \mu\text{Ci}$ of $[1\text{-}^{14}\text{C}]$ -glycollate or $0.5\ \mu\text{Ci}$ of $[2\text{-}^{14}\text{C}]$ glycollate, was incubated under the conditions described in the Materials and Methods section, with 0.1 ml of a spinach-beet peroxisomal preparation in 33 mM- K_2HPO_4 -citric acid buffer (●), or in 33 mM-glycylglycine-KOH buffer (■).

Table 4. Effect of various reagents on glycollate decarboxylation by a spinach-beet peroxisomal preparation

Details of incubation conditions are given in the Materials and Methods section.

Reagent	Rate of glycollate decarboxylation	
	(μmol of CO_2 released/h per mg of protein)	(% of rate without reagent)
None	0.77	100
None (omit FMN)	0.59	77
1 mM-Formate	0.87	113
1 mM-Glycine	0.72	94
1 mM-EDTA	0.87	113
5 mM-Glyoxylate	0.25	32
1 mM-Oxalate	0.77	100
1 mM-Azide	8.25	1071
17 mM-3-Amino-1,2,4-triazole	8.87	1152
1 mM-Pyrid-2-yl- α -hydroxymethanesulphonate	0	0
5 mM-L-Glutamate	0.60	78

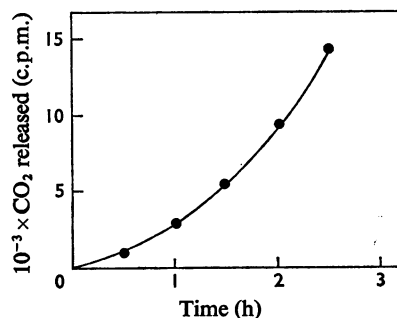


Fig. 4. Time-course of $^{14}\text{CO}_2$ release from $[1\text{-}^{14}\text{C}]$ glycollate by a spinach-beet peroxisomal preparation at pH 8

The reaction conditions are described in the Materials and Methods section.

at 100°C . In a typical test assay, 36% of the glycollate had been used up, of which 29% remained as glyoxylate in the reaction mixture, 3.8% was released as CO_2 and 3.2% was converted into oxalate. Thus most of the H_2O_2 arose from the oxidation of glycollate to glyoxylate; the secondary conversion of glyoxylate into oxalate made little contribution. In support of this, 1 mM-oxalate did not inhibit glycollate decarboxylation (Table 4), whereas it does inhibit that of glyoxylate (Table 2). Oxalate, at this concentration, inhibits glycollate oxidase only when glyoxylate is the substrate (Richardson & Tolbert, 1961). Whereas the apparent K_m value of glycollate oxidase for glyoxylate is 5.4 mM, that for glycollate is 0.38 mM, which would account for the observation that 5 mM-glycollate was sufficient to saturate the glycollate decarboxylation by peroxisomes.

Release of CO_2 from glycollate and glyoxylate by spinach preparations

Kisaki & Tolbert (1969) reported that peroxisomes isolated from spinach leaves released no CO_2 from glycollate and glyoxylate and argued that the catalase present in peroxisomes was sufficiently active to destroy any H_2O_2 produced during glycollate oxidation. However, when the assay methods developed for spinach-beet peroxisomes were applied to spinach peroxisomes prepared as described by the above authors, the rates of $^{14}\text{CO}_2$ release from $[1\text{-}^{14}\text{C}]$ glyoxylate and $[1\text{-}^{14}\text{C}]$ glycollate were up to 5 and $7\ \mu\text{mol/h}$ per mg of protein respectively at pH 8. These peroxisome fractions contained no chlorophyll, but traces of cytochrome oxidase activity suggested mitochondrial contamination. However, the activity could not be attributed to mitochondria since the mitochondrial fraction from the sucrose gradients showed little ability to decarboxylate either glycollate or glyoxylate under these conditions. Indeed, the

Table 5. *Effect of amino acids on glycollate decarboxylation by spinach peroxisomes at pH 8*

The reaction was carried out under the conditions described in the Materials and Methods section.

Reagent added	Rate of glycollate decarboxylation (μmol of CO_2 released/h per ml of preparation)
None	0.27
0.1 mM-Pyridoxal phosphate	0.21
0.1 mM-Pyridoxal phosphate + 20 mM-L-serine	0.14
0.1 mM-Pyridoxal phosphate + 20 mM-L-glutamate	0.21
0.1 mM-Pyridoxal phosphate + 20 mM-L-serine + 20 mM-L-glutamate	0.11

release of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glycollate or $[1-^{14}\text{C}]$ glyoxylate catalysed by spinach peroxisomes was stimulated by catalase inhibitors and by added H_2O_2 , and decreased by an excess of catalase. It is clear that the same reactions identified for spinach-beet preparations are also present in spinach. Addition of sufficient pyridoxal phosphate and amino acids to allow glycine formation to proceed at its maximum rate (Rehfeld & Tolbert, 1972) decreased glycollate decarboxylation, but by no means abolished it (Table 5). Similarly, added glutamate decreased only slightly glycollate decarboxylation by spinach-beet peroxisomes (Table 4).

In the assays of Kisaki & Tolbert (1969), isotopically-labelled substrate without added carrier was used. Since enough H_2O_2 must be generated by the oxidation of the substrate for decarboxylation to occur, it seems likely that the substrate concentration used in their experiments was insufficient.

Discussion

At low pH values, peroxisomal fractions from spinach-beet leaves catalysed the release of CO_2 from both carbon atoms of glyoxylate, although more rapidly from the carboxyl group. However, these rates would be limited *in vivo* by the slow rate of glycollate oxidation below pH 6 (Fig. 3). Since little CO_2 is released from the α -carbon atom of glycollate during photorespiration (Zelitch, 1966), it seems unlikely that glyoxylate decarboxylation at pH 5 plays a major role in photorespiration.

At pH 8, peroxisomes catalysed little $^{14}\text{CO}_2$ release from $[2-^{14}\text{C}]$ glycollate or $[2-^{14}\text{C}]$ glyoxylate, but considerable release from the carboxyl groups of these compounds. It might be objected that high concentrations of glyoxylate and glycollate are required to achieve these rates. A comparison of the known rates of photorespiration with the glycollate

oxidase activities of leaf extracts (Zelitch, 1971) shows that glycollate oxidase *in vivo* must often be working close to its maximum velocity, and hence would be nearly saturated with substrate. Further, the glutamate-glyoxylate aminotransferase, which is the main enzyme catalysing the conversion of glyoxylate into glycine in peroxisomes, has a K_m value for glyoxylate of 4.4 mM (Kisaki & Tolbert, 1969). The decarboxylation of glyoxylate in peroxisomes was observed to compete successfully at pH 8, even when glycine was formed from glyoxylate at the maximum rate, the aminotransferases being saturated with their amino donors.

For both glycollate and glyoxylate, decarboxylation at pH 8 is due to the generation of H_2O_2 by the action of glycollate oxidase. Although most of the H_2O_2 was destroyed by catalase, enough descape decomposition to allow significant rates of non-enzymic glyoxylate decarboxylation. Even when a large excess of catalase was added, this reaction was not completely suppressed. There are other observations on extracts and cells which suggest the incomplete destruction of H_2O_2 by catalase. Boveris *et al.* (1972) demonstrated the escape of measurable quantities of H_2O_2 into the surrounding medium when isolated rat-liver peroxisomes oxidized D-alanine or urate. Catalase has been shown to be incompletely effective in the protection of haemoglobin in erythrocytes against steady rates of H_2O_2 generation (Keilin & Hartree, 1945; Cohen & Hochstein, 1963). Chance (1952) showed that catalase is converted into an inactive form under conditions of continuous H_2O_2 generation. The activity of catalase in leaf peroxisomes *in vitro* and *in vivo* may thus be insufficient to prevent the attack by peroxide on glyoxylate.

The direct decarboxylation of glyoxylate could therefore occur in leaf peroxisomes *in vivo* and make some contribution to photorespiration. In the experiments reported here, the rate of glycollate decarboxylation was less than 15% of the glycollate oxidase activity of the peroxisomes. The glycollate oxidase of typical leaf homogenates catalysed the production of $60\mu\text{mol}$ of glyoxylate/h per mg of chlorophyll, so that the glycollate decarboxylation in peroxisomes might contribute about $10\mu\text{mol}$ of CO_2 /h per mg of chlorophyll to photorespiration. As rates of photorespiration up to $100\mu\text{mol}$ of CO_2 produced/h per mg of chlorophyll have been recorded (see Zelitch, 1971), it would appear at first sight that the peroxisomal decarboxylation of glycollate provides only a minor contribution to CO_2 release during photorespiration. However, the contribution of this reaction would depend especially upon the activity of catalase *in vivo*. If the conversion of catalase into an inactive form occurs *in vivo*, higher rates of glyoxylate decarboxylation than those reported here would be expected, for it is shown that

the inhibition of catalase increased the rate of glycolate decarboxylation tenfold (Table 3). Under these conditions a major contribution to photorespiration from this oxidation would be expected. Any more exact correlation requires the measurement together of both the photorespiration of leaves and the decarboxylation activity of the peroxisomes extracted from them.

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