JOURNAL OF BACTERIOLOGY, Nov. 1986, p. 702–707
0021-9193/86/110702-06$02.00/0

Vol. 168, No. 2

Glyphosate Catabolism by *Pseudomonas* sp. Strain PG2982

DEAN L. SHINABARGER, AND HUGH D. BRAYMER*

Department of Microbiology and Agricultural Experiment Station, Louisiana State University, Baton Rouge, Louisiana 70803

Received 9 April 1986/Accepted 2 July 1986

The pathway for the degradation of glyphosate (*N*-phosphonomethylglycine) by *Pseudomonas* sp. PG2982 has been determined by using metabolic radiolabeling experiments. Radiorespirometry experiments utilizing [3-^{14}C]glyphosate revealed that approximately 50 to 59% of the C-3 carbon was oxidized to CO_2. Fractionation of stationary-phase cells labeled with [3-^{14}C]glyphosate revealed that from 45 to 47% of the assimilated label is distributed to proteins and that the amino acids methionine and serine are highly labeled. Adenine and guanine received 90% of the C-3 label found in the nucleic acid fraction, and the only pyrimidine base labeled was thymine. These results indicated that C-3 of glyphosate was at some point metabolized to a C-1 compound whose ultimate fate could be both oxidation to CO_2 and distribution to amino acids and nucleic acid bases that receive a C-1 group from the C-1-donating coenzyme tetrahydrofolate. Pulse-labeling of PG2982 cells with [3-^{14}C]glyphosate resulted in the isolation of [3-^{14}C]sarcosine as an intermediate in glyphosate degradation. Examination of crude extracts prepared from PG2982 cells revealed the presence of a sarcosine-oxidizing enzyme that oxidizes sarcosine to glycine and formaldehyde. These results indicate that the first step in glyphosate degradation by PG2982 is cleavage of the carbon-phosphorus bond, resulting in the release of sarcosine and a phosphate group. The phosphate group is utilized as a source of phosphorus, and the sarcosine is degraded to glycine and formaldehyde. This pathway is supported by the results of [1,2-^{14}C]glyphosate metabolism studies, which show that radioactivity in the proteins of labeled cells is found only in the glycine and serine residues.

Glyphosate (*N*-phosphonomethylglycine) is the active ingredient in the herbicide Roundup (Fig. 1). The primary mode of action of glyphosate is the inhibition of 3-phosphoshikimate-1-carboxyvinyl transferase, an enzyme of the shikimate pathway responsible for the biosynthesis of aromatic amino acids in bacteria and plants (6, 12). Recent studies with glyphosate-resistant *Salmonella typhimurium* mutants (2) and recombinant *Escherichia coli* strains containing plasmids that overproduce 3-phosphoshikimate-1-carboxyvinyl transferase (9) have confirmed that glyphosate is a potent inhibitor of this enzyme.

There have been several studies investigating the mode of action of glyphosate and the development of glyphosate resistance in bacteria and plants. This is in contrast to the study of glyphosate metabolism, which has been hampered by the lack of pure degradative bacterial cultures. The fate of glyphosate in soil has been reported to be complete degradation by soil microbes (8, 10, 13). However, these studies yield little information about the pathway of glyphosate metabolism utilized by individual bacterial species.

We previously reported the isolation of a thiamine-requiring *Pseudomonas* sp. PG2982 from a stock culture of *Pseudomonas aeruginosa* ATCC 9027 that can utilize glyphosate as a sole phosphorus source (7). In batch culture this organism cmpletely utilizes 1.0 mM glyphosate, yielding a cell density equal to that obtained with an equimolar concentration of P_i (7). Recently, Jacob et al. (5) used solid-state ^{13}C and ^{15}N nuclear magnetic resonance experiments to study the metabolism of glyphosate by PG2982. These investigators reported that glyphosate is cleaved directly to glycine, and that this glycine is utilized by PG2982 for protein and nucleic acid biosynthesis. However, their study did not indicate whether the organism breaks the carbon-phosphorus bond of glyphosate before or after glycine is released to obtain phosphorus for growth.

To determine the pathway for glyphosate degradation by PG2982, we performed radiotracer experiments with ^{14}C-labeled glyphosate. These experiments have resulted in the successful isolation of an intermediate of glyphosate degradation in sufficient quantities for chromatographic and enzymatic analysis. Subsequent studies with this metabolite and radiorespirometry experiments with [3-^{14}C]glyphosate have led to the formulation of a pathway of glyphosate degradation in which cleavage of the C-P bond is the first step.

MATERIALS AND METHODS

Organism and culture conditions. PG2982 is a glyphosate-degrading *Pseudomonas* sp. isolated in our laboratory (7). The medium used for the maintenance and growth of PG2982 has been described in a previous report (7). Cultures were routinely grown in 300-ml sidearm flasks at 32°C on an orbital shaker (250 rpm). Cultures for experiments were always started with a 1% log-phase inoculum.

Chemicals and radiochemicals. The free acid form of glyphosate (99.7% purity) and [3-^{14}C]glyphosate (specific activity, 9.3 mCi mmol^{-1}) were gifts from Monsanto Co., St. Louis, Mo. [U-^{14}C]glycine (specific activity, 100 mCi mmol^{-1}), [^{14}C]formaldehyde (specific activity, 40 mCi mmol^{-1}), and [^{14}C]formate (specific activity, 44 mCi mmol^{-1}) were obtained from Research Products International Corp. (Mount Prospect, Ill.). Chloromethylphosphonic acid was purchased from Aldrich Chemical Co., Milwaukee, Wis. All other chemicals used in this study were of reagent grade.

Analytical methods. Culture turbidity was measured with a Klett-Summerson colorimeter and a red filter. Bacterial dry weight measurements were made by filtering cells (with a 0.45-μm membrane filter) and washing them with 10 ml of Dworkin-Foster salts solution (7). The membrane filter was

* Corresponding author.

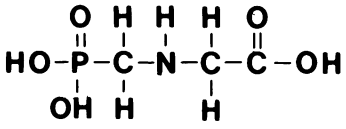

FIG. 1. Structure of glyphosate.

dried for 6 h in an oven at 60°C and weighed. Glyphosate and other amine-containing metabolites were assayed on a Beckman amino acid analyzer, model 120C, by the procedure of Moore et al. (7). Formaldehyde was determined by the method of Nash as described by Wood and Gest (14). Unless indicated otherwise, radioactivity was determined by adding a portion of the sample to be assayed to 5 ml of Aquasol scintillation cocktail (New England Nuclear Corp., Boston, Mass.) and counting in a Beckman LS 6800 scintillation counter.

Radiorespirometry experiments. To determine whether [3-^{14}C]glyphosate was oxidized to CO_2 by PG2982, 300-ml sidearm flasks were fitted with a CO_2 trap with the apparatus described by Dobrogosz (3). The cultures were incubated at 32°C on an orbital shaker (250 rpm) and allowed to reach the stationary phase. During the course of the experiment, a 250-μl sample of each culture was removed with a sterile syringe fitted with a 10-cm (22-gauge) needle that had been inserted through a rubber septum in the culture flask. The culture samples were treated as follows. (i) Duplicate 100-μl portions were filtered, washed, and dried as described above. These samples were weighed for bacterial dry weight measurements, and the radioactivity was counted in scintillation vials as assimilated [^{14}C]glyphosate. (ii) Duplicate 10-μl portions of the culture filtrate obtained above were counted to determine the radioactivity remaining in the extracellular medium. To determine whether [^{14}C]formaldehyde or [^{14}C]formate was oxidized to CO_2, mid-log-phase PG2982 cells (10 ml) growing in glyphosate broth were harvested by centrifugation, washed once with Dworkin-Foster salts solution, and suspended in 10 ml of glyphosate broth. A sample of 0.5 μCi of [^{14}C]formaldehyde or [^{14}C]formate was added to the culture, and CO_2 was trapped as outlined above.

Fractionation of labeled cells. Mid-log-phase PG2982 cells growing in medium containing either [3-^{14}C]glyphosate or [1,2-^{14}C]glyphosate were harvested and washed twice with Dworkin-Foster salts solution before fractionation by a modified Roberts technique (3). Duplicate samples were counted for each fractionation step.

Distribution of radioactivity in nucleic acid bases. The nucleic acid fraction obtained from the modified Roberts procedure was precipitated with ethanol to obtain the nucleic acids. This precipitate was hydrolyzed with 12 N perchloric acid, and the bases were separated by thin-layer chromatography on plastic-backed cellulose sheets (1). The bases were visualized under UV light and scraped into scintillation vials for counting.

Distribution of radioactivity in proteins. The protein fraction obtained from the modified Roberts procedure was precipitated and washed twice with 6 N HCl. This precipitate was then hydrolyzed in 6 N HCl at 110°C for 24 h. A sample of this hydrolysate was injected into a Beckman 120C amino acid analyzer for quantitative determination of amino acid content, and a second sample was chromatographed by two-dimensional thin-layer chromatography on plastic-backed cellulose plates (20 by 20 cm, 0.1-mm layer) with isopropanol-formic acid-water (20:1:5, vol/vol/vol) in the first direction and butanol-acetone-diethylamine-water (30:30:6:15, vol/vol/vol/vol) in the second direction. Radioactive amino acids were located by scraping the spots on the plate where standard amino acids migrated and counting in liquid scintillation vials.

Radiotracer experiments. Mid-log-phase cells (20 ml) grown in glyphosate broth were centrifuged at 12,000 × *g* for 10 min at room temperature and washed once with Dworkin-Foster salts solution. The cell pellet was suspended in 5 ml of Dworkin-Foster salts solution containing 10 μCi of [3-^{14}C]glyphosate (specific activity, 9.3 mCi mmol^{-1}) and incubated at 32°C on an orbital shaker for 1 h. The cells were harvested by centrifugation and extracted two times with 0.5-ml portions of 10% trichloroacetic acid. The trichloroacetic acid extracts were concentrated under reduced pressure and suspended in 0.25 ml of 0.2 N sodium citrate buffer.

Purification of ^{14}C-labeled metabolites. Fractionation of glyphosate and intermediary metabolites was carried out with a Beckman 120C amino acid analyzer modified so that fractions could be collected directly from the ion-exchange column (W1-type resin) of the amino acid analyzer without passing through the ninhydrin mixing chamber. Fractions (1 ml) were collected and assayed by liquid scintillation as described above. Fractions corresponding to each radioactive peak were pooled and passed through a 1- by 15-cm column of Dowex 50W-X8 (20–50 mesh, H^+ form; Bio-Rad Laboratories, Richmond, Calif.). The sodium citrate buffer from the amino acid analyzer and [^{14}C]glyphosate were eluted with 100 ml of water, whereas bound metabolites were eluted with 25 ml of 2.0 N NH_4OH. The NH_4OH wash was concentrated under reduced pressure and assayed by thin-layer chromatography on cellulose sheets (20 by 20 cm, 100-μm layer) with the following solvent systems: methanol-pyridine-hydrochloric acid-water (80:20:2:18, vol/vol/vol/vol), ethanol–water–17 N ammonium hydroxide–trichloroacetic acid–15 N acetic acid (55:35:2.5:3.5:2, vol/vol/vol/wt/vol), isopropanol-formic acid-water (20:1:5, vol/vol/vol), sec-butanol–formic acid–water (70:10:20, vol/vol/vol), and *n*-butanol–acetic acid–water (30:7.5:12.5, vol/vol/vol).

Identification of sarcosine. The ^{14}C-labeled metabolite identified as sarcosine by both ion-exchange and thin-layer chromatography was further analyzed by treatment with the enzyme sarcosine oxidase (sarcosine:oxygen oxidoreductase [demethylating] EC 1.5.3.1). The assay system consisted of a sample containing 3,000 cpm of labeled material in 60 μl, 1 ml of 50 mM potassium phosphate buffer (pH 7.5), and 1 U of sarcosine oxidase. The reaction was carried out at 37°C in a 10- by 65-mm glass tube sealed with a serum stopper. After a 30-min incubation, 2 ml of a solution containing 0.2% 2,4-dinitrophenylhydrazine in 2 N HCl was injected through the serum stopper. Unlabeled formaldehyde (2.0 μmol) was then injected into the tube to act as a carrier for the precipitation of any [^{14}C]formaldehyde formed during the reaction. After 30 min of incubation at room temperature, the yellow precipitate formed in the tube was collected on a 0.45-μm filter and washed with 10 ml of 2 N HCl. The yellow precipitate was dissolved in ethyl acetate and chromatographed on silica gel plates (20 by 20 cm, 100 μm, AllTech Associates, Inc.) with three different solvent systems (15): chloroform-acetone (9:1, vol/vol), benzene, and chloroform-diethyl ether (8:2, vol/vol). Radioactivity on these plates was located by cutting the thin-layer plate into 1.0-cm squares and dropping each square into a vial containing 5.0 ml of scintillation fluid. The migration of radioactive compounds on the plates was compared with the migration

of the 2,4-dinitrophenylhydrazone derivatives of formaldehyde and other aldehydes.

Synthesis and purification of glyphosate. Glyphosate was synthesized by the procedure of Fredericks and Summers (4) for the synthesis of aminomethylphosphonic acids related to glyphosate. Briefly, 0.2 mol of glycine and 0.02 mol of chloromethylphosphonic acid were dissolved in 50 ml of water, adjusted to pH 10.5 with 8 M NaOH, and refluxed for 24 h. The crude product was precipitated from the reflux mixture with absolute ethanol, suspended in 0.2 N sodium citrate buffer, and fractionated with a Beckman 120C amino acid analyzer as described above. For the synthesis of [1,2-^{14}C]glyphosate, 100 μCi of [U-^{14}C]glycine (specific activity, 20 mCi mmol^{-1}) was refluxed with 4 μmol of chloromethylphosphonic acid. The 24-h reflux mixture was concentrated to 1 ml under reduced pressure and applied to the Beckman 120C amino acid analyzer column. Liquid scintillation assay of the resultant fractions gave two radioactive peaks (I and II) eluting at fractions 5 through 7 and 17 through 19. Control experiments indicated that glyphosate eluted at fractions 5 through 7. The peak I sample was applied to a 30- by 1.5-cm column containing AG1-X8 (100-200 mesh, chloride form; Bio-Rad). The [1,2-^{14}C]glyphosate was eluted with 150 ml of water, whereas the sodium citrate buffer was retained by the column. This water wash was concentrated under reduced pressure and chromatographed on cellulose plates by the method of Sprankel et al. (11). Radioactivity on the plates was located by cutting the plates into 1 cm squares and counting by liquid scintillation assay. The yield of glyphosate under these conditions represented 25 to 30% of the theoretical yield. With this purification scheme, no [^{14}C]glycine was detected in the final product.

Enzyme assays. Crude cell-free extracts for enzyme assays were prepared from washed PG2982 cells grown under various conditions. In all cases the cells were pelleted at 12,000 × *g* and washed twice with Dworkin-Foster salts solution before suspension in 50 mM Tris hydrochloride buffer (pH 7.0). The cells were broken by three 30-s sonications in an ice bath. The extracts were centrifuged at 20,000 × *g* (4°C) for 20 min to sediment unbroken cells and cell wall material, and the supernatant was collected and kept on ice until the assays were run (always within 30 min). The assay for sarcosine oxidase contained 0.2 ml of the crude extract, 0.6 ml of 50 mM Tris hydrochloride (pH 7.0), and 0.1 ml of 0.5 M sarcosine. The reagents were added in this order and incubated at 30°C for 40 min. The reaction was stopped by the addition of 0.2 ml of 10% trichloroacetic acid and centrifuged at 8,740 × *g* in a microfuge to sediment the protein. Portions of the supernatant were injected into a Beckman 120C amino acid analyzer for glycine determination.

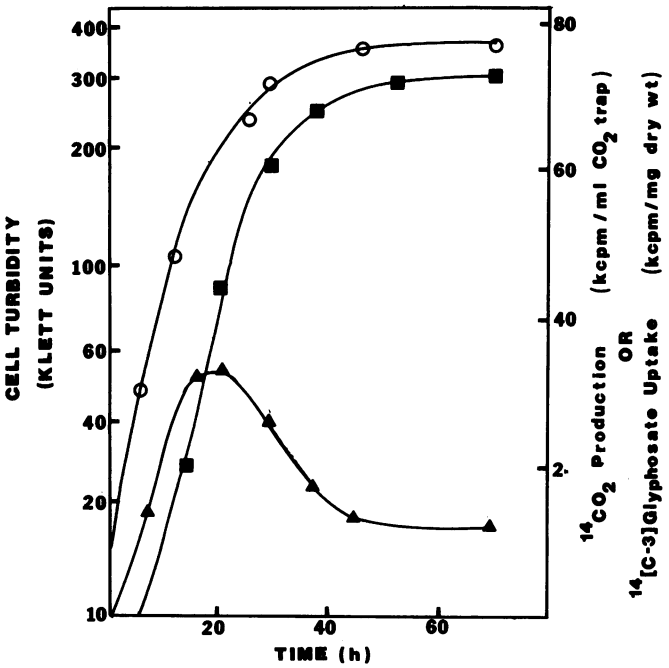

FIG. 2. [3-^{14}C]glyphosate uptake (▲) and CO_2 production (■) by PG2982 cells grown in batch culture. The minimal medium contained 5 μCi of [3-^{14}C]glyphosate (0.5 mM final concentration) as the sole phosphorus source. Cell growth (○) was monitored with a Klett-Summerson colorimeter.

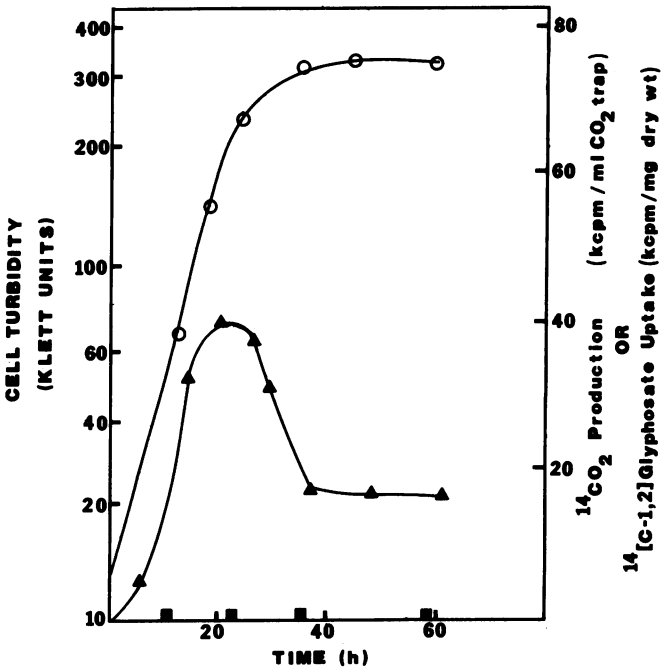

FIG. 3. [1,2-^{14}C]glyphosate uptake (▲) and CO_2 production (■) by PG2982 cells grown in batch culture. The minimal medium contained 1 μCi of [3-^{14}C]glyphosate (0.5 mM final concentration) as the sole phosphorus source. Cell growth (○) was monitored with a Klett-Summerson colorimeter.

RESULTS

Utilization of glyphosate by PG2982. PG2982 cells began oxidizing the C-3 carbon of glyphosate to CO_2 early in the logarithmic phase of growth (Fig. 2). Data obtained from several independent experiments have shown that 50 to 59% of the C-3 carbon of glyphosate is found in the CO_2 trap at the end of batch growth. Radioactivity was assimilated by whole cells (Fig. 2) until the midlog phase, after which time the amount of label assimilated per milligram of bacterial dry weight decreased dramatically.

There was no detectable CO_2 production from C-1 or C-2 of glyphosate (Fig. 3). Instead there was a steady assimilation of radioactivity by PG2982 cells throughout the growth cycle until the stationary phase was reached. Approximately

TABLE 1. Distribution of radioactivity in PG2982 cells after incubation with [^{14}C]glyphosate and fractionation by the modified Robert technique

Fraction	Recovery (%) of label	
	[3-^{14}C]glyphosate	[1,2-^{14}C]glyphosate
Metabolic pool	3–5	1–2
Lipid	20–23	10–13
Nucleic acid	24–26	22–24
Protein	45–47	68–71

100% of the label from [1,2-^{14}C]glyphosate was assimilated by whole cells, whereas less than 1% of the original radioactivity remained in the culture supernatant.

Fractionation of labeled cells. PG2982 cells labeled with [3-^{14}C]glyphosate contained approximately 50% of the radioactivity in the protein and cell wall fraction; the remainder of the assimilated label was evenly incorporated into the nucleic acid (24 to 26%) and lipid (20 to 23%) pools (Table 1). Only 3 to 5% of the assimilated radioactivity could be found in the amino acid-organic acid pool (metabolic pool).

Fractionation of PG2982 cells after incubation with [1,2-^{14}C]glyphosate revealed that 1% of the label remained in the metabolic pools, 10 to 13% was incorporated into lipids, 24% was found in the nucleic acid fraction, and 68 to 71% was found in the protein and cell wall fraction (Table 1).

Distribution of radioactivity in nucleic acid bases. Analysis of the component nucleic acid bases from cells grown in the presence of [1,2-^{14}C]glyphosate revealed that all of the radioactivity was in the purine bases adenine and guanine (Table 2). No detectable radioactivity was found in the pyrimidine bases of these cells. The nucleic acid bases adenine and guanine were labeled in cells incubated with [3-^{14}C]glyphosate (Table 2). Only one of the pyrimidine bases analyzed from these cells, thymine, contained radioactivity.

Assimilation of [^{14}C]glyphosate into proteins. Acid hydrolysis and subsequent chromatographic analysis of the protein fraction from [1,2-^{14}C]glyphosate-labeled cells revealed that glycine and serine were the only amino acids that received radioactivity. The specific activity of glycine (5.2 cpm nmol^{-1}) was more than two times greater than that of serine (2.4 cpm nmol^{-1}). Analysis of the protein hydrolysate from [3-^{14}C]glyphosate-labeled cells showed that methionine and serine contained the protein-associated radioactivity, with methionine (17.6 cpm nmol^{-1}) receiving nearly three times more label than serine (6.7 cpm nmol^{-1}).

Analysis of intermediates. Fractionation of the trichloroacetic acid extract from PG2982 cells incubated with [3-^{14}C]glyphosate gave two radioactive peaks (I and II) eluting at fractions 5 through 7 and 13 through 16, respectively (Fig. 4). Control experiments indicated that [^{14}C]glyphosate eluted at the former volume. Preliminary experiments indicated that the size of peak II increased greatly when the cells were incubated with [3-^{14}C]glyphosate in the presence of 50 mM sarcosine. Peak II was further purified by passage through a Dowex 50W-X4 column as outlined above, and the radioactive material that was eluted in the NH_4OH wash was analyzed by thin-layer chromatography. The radioactive compound purified by this procedure migrated with sarcosine in all five solvent systems tested.

TABLE 2. Distribution of radioactivity in nucleic acid bases from PG2982 cells incubated in the presence of [^{14}C]glyphosate

Base	% of total radioactivity in nucleic acid base hydrolysate	
	[3-^{14}C]glyphosate	[1,2-^{14}C]glyphosate
Adenine	40	37
Guanine	51	63
Cytosine	0	0
Thymine	9	0
Uracil	0	0

Incubation of the ^{14}C-labeled metabolite with the enzyme sarcosine oxidase revealed that a radioactive product was produced during the reaction which could be precipitated by 2,4-dinitrophenylhydrazine. This product was identified as the 2,4-dinitrophenylhydrazone derivative of [^{14}C]formaldehyde by thin-layer chromatography with three different solvent systems. Radioactivity associated with the precipitated product migrated with authentic 2,4-dinitrophenylhydrazone formaldehyde on each silica gel plate.

Utilization of formate and formaldehyde by PG2982. Incubation of logarithmic-phase PG2982 cells with either [^{14}C]formaldehyde or [^{14}C]formate resulted in the rapid production of CO_2 after a 20-min lag period (Fig. 5). Approximately 66% of the formaldehyde and 100% of the formate added were oxidized to CO_2 after 7 h of incubation. There was no radioactivity in CO_2 traps from uninoculated control flasks.

Sarcosine oxidation enzyme assays. There was a sarcosine-degrading activity present in crude extracts prepared from cells grown on glyphosate as the sole phosphorus source. Glycine and formaldehyde are the products of this reaction, with 1 mol of glycine produced per mol of sarcosine utilized. This enzyme remained active after dialysis overnight in 50 mM Tris hydrochloride buffer (pH 7.0) and did not require the addition of cofactors or coenzymes. A sarcosine oxidase activity of 1.84 nmol min^{-1} mg of protein^{-1} was measured in crude extracts prepared from cells grown with glyphosate as the phosphorus source, whereas cells utilizing inorganic phosphate as a phosphorus source had a much lower activity of 0.30 nmol min^{-1} mg of protein^{-1}. PG2982 cells utilizing sarcosine as a sole source of nitrogen had the highest sarcosine oxidase activity (4.88 nmol min^{-1} mg of protein^{-1}).

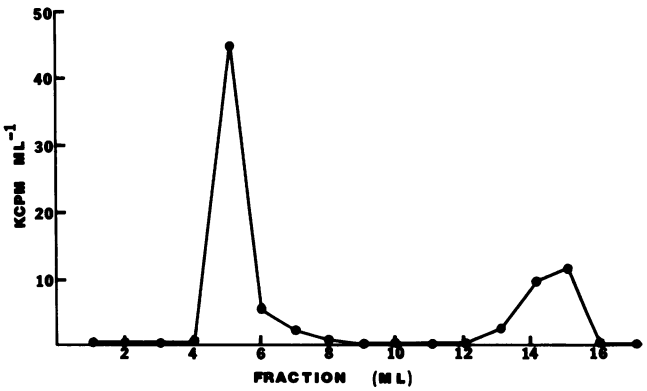

FIG. 4. Fractionation of trichloroacetic acid extract from PG2982 cells incubated with [3-^{14}C]glyphosate for 1 h. The extract was injected into a Beckman 120C amino acid analyzer, and fractions (1 ml) were collected directly from the ion-exchange column (W1-type resin) of the analyzer. The peak represented by fractions 5 through 7 is [3-^{14}C]glyphosate, and the later peak (fractions 13 through 15) is [3-^{14}C]sarcosine.

DISCUSSION

The results of this report indicate that *Pseudomonas* sp. PG2982 cleaves the carbon-phosphorus bond of glyphosate, resulting in the release of a phosphate group and a molecule of sarcosine (Fig. 6). The sarcosine is cleaved by a sarcosine-oxidizing enzyme present in PG2982 to glycine and formaldehyde, and this glycine is used for the biosynthesis of proteins and purine bases. Approximately 50% of the formaldehyde derived from glyphosate catabolism enters into tetrahydrofolate-mediated reactions, whereas the other 50% is oxidized to CO_2.

Evidence for cleavage of the C-P bond in glyphosate by PG2982 initially came from radiorespirometry studies performed in this laboratory (K. Moore, M.S. thesis, Louisiana State University, Baton Rouge, 1983). These studies, along with radiorespirometry data from this report, show that the phosphorus-bonded carbon of glyphosate is oxidized to CO_2 as the organism utilizes glyphosate as a sole source of phosphorus. However, the distribution of [3-^{14}C]glyphosate into various molecular fractions of PG2982 indicates that the C-3 carbon is not initially released from glyphosate as CO_2. For radioactivity from [3-^{14}C]glyphosate to be distributed into all of the cellular fractions examined by the modified Robert technique, the C-3 carbon of glyphosate must somehow be converted to a form which can react with tetrahydrofolate. This coenzyme is responsible for the transfer of single-carbon compounds in a variety of cellular reactions such as the addition of carbon to positions 2 and 8 on the purine ring and biosynthesis of the amino acids methionine and serine. Formaldehyde and formate are single-carbon compounds which can react with tetrahydrofolate, and both of these compounds can be oxidized to CO_2

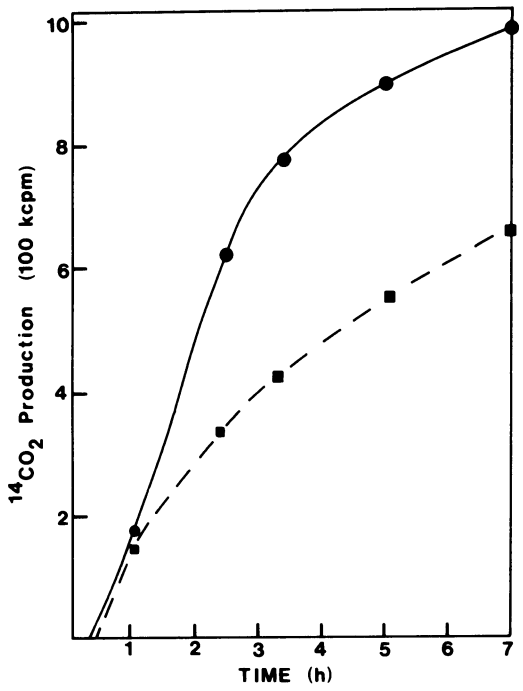

FIG. 5. CO_2 production from [^{14}C]formate (●) and [^{14}C]formaldehyde (■) by PG2982 cells. Mid-log-phase PG2982 cells growing in glyphosate broth were harvested by centrifugation, washed, and assayed for the production of $^{14}CO_2$.

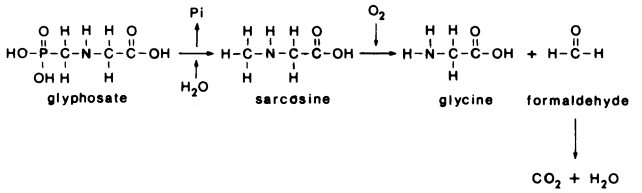

FIG. 6. Proposed pathway for glyphosate degradation by *Pseudomonas* sp. GP2982.

by PG2982. Our evidence indicates that C-3 of glyphosate is released as a formaldehyde molecule when [3-^{14}C]sarcosine derived from [3-^{14}C]glyphosate is cleaved by the sarcosine-oxidizing enzyme of PG2982. This formaldehyde can then react with tetrahydrofolate to be used for the biosynthesis of methionine and serine as well as adenine, guanine, and thymine (Table 2). Approximately 50% of this formaldehyde is oxidized to CO_2. We do not know at this time whether formaldehyde is converted to formate before being oxidized to CO_2. However, if this were the case formate could still react with tetrahydrofolate and also be oxidized to CO_2. The distribution of radioactivity into molecular fractions of PG2982 would be similar under these conditions.

The distribution of [1,2-^{14}C]glyphosate in PG2982 after fractionation by the modified Robert technique was very similar to that found by Jacob et al. (5) in their study with [1-^{13}C]glyphosate. We found that proteins and cell wall material received approximately 70% of the label, and that the amino acids glycine and serine contained most of the radioactivity. Analysis of the nucleic acid bases revealed that only the purines were labeled, which is exactly what Jacob et al. (5) found in their study. Glycine contributes to purine biosynthesis by providing C-4 and C-5 as well as the nitrogen at position 7 of the purine ring. Although we did not determine the position of the label in the purine bases, the absence of radioactivity in the pyrimidine bases along with the extensive labeling of glycine and serine residues in proteins of PG2982 supports a pathway of glyphosate metabolism in which the glycine moiety is released intact from the glyphosate molecule.

Jacob et al. have published the only other report in which glyphosate metabolism by a pure culture has been investigated (5). They performed solid-state nuclear magnetic resonance experiments to determine the ultimate fate of the carbon and nitrogen atoms of glyphosate in PG2982 cells. These investigators have proposed a pathway for glyphosate degradation in which glyphosate is cleaved directly to glycine. However, since no intermediates were found in their study, Jacob et al. could not determine the product of the first step in the metabolism of glyphosate. Based on the isolation of [3-^{14}C]sarcosine, the presence of a sarcosine-oxidizing activity in crude extracts of PG2982, and the labeling patterns obtained in this laboratory, we propose that C-P bond cleavage is the first step in the degradation of glyphosate by PG2982. This results in cleavage of glyphosate directly to sarcosine, which is then degraded to formaldehyde and glycine. The ability of PG2982 to oxidize formaldehyde and formate to CO_2 lends support to our proposed pathway. Studies with soil microorganisms have shown that aminomethylphosphonic acid, not sarcosine, is the major metabolic intermediate of glyphosate degradation in soil (10). Therefore it appears that the glyphosate degradation pathway used by PG2982 is unique to ths pure culture.

The successful isolation of [3-^{14}C]sarcosine as an intermediate in the degradation of glyphosate by PG2982 required the addition of unlabeled sarcosine to the suspension medium. This technique is similar to the pulse-chase experiments used to study metabolic pathways in which the concentration of intermediates is low. In our study, the high concentration of sarcosine in the resuspension medium served to increase the concentration of sarcosine in the cellular pool. This presumably resulted in an intracellular sarcosine concentration that was great enough to prevent the metabolism of all of the [3-^{14}C]glyphosate-derived [3-^{14}C]sarcosine.

[^{14}C]glycine has been detected in cells pulsed with [1,2-^{14}C]glyphosate synthesized in our laboratory (data not shown). However, this required the presence of chloramphenicol to stop protein synthesis, thereby allowing sufficient (1,2-^{14}C]glycine to accumulate to be detected. The other intermediates of glyphosate degradation by PG2982, formaldehyde and possibly formate, are either oxidized to CO_2 or reacted with tetrahydrofolate.

Sarcosine oxidation by PG2982 appears to be regulated by sarcosine levels in the cell. This is evident by an over 2.5-fold increase in the specific activity of sarcosine oxidase when cells are using sarcosine as a sole source of nitrogen. When the culture is using P_i as a phosphorus source in the absence of sarcosine, sarcosine oxidase activity is very low. However, when glyphosate replaces P_i as the sole phosphorus source, there is a threefold increase in enzyme activity. This increase in enzyme activity is thought to result from the degradation of glyphosate to sarcosine by PG2982.

We do not know at this time whether the first step in glyphosate degradation, C-P bond cleavage, is an inducible or constitutive activity. We are currently examining crude extracts of PG2982 for C-P bond cleavage activity by using [^{14}C]glyphosate.

ACKNOWLEDGMENTS

J. Fitzgibbon provided helpful advice concerning the synthesis of glyphosate. T. Stelling and S. Schumacher provided valuable nuclear magnetic resonance work to confirm the identity of glyphosate.

LITERATURE CITED

1. **Bendich, A.** 1957. Methods for characterization of nucleic acids by base composition. Methods Enzymol. **3**:715–723.
2. **Comai, L., L. C. Sen, and D. M. Stalker.** 1983. An altered aroA gene product confers resistance to the herbicide glyphosate. Science **221**:370–371.
3. **Dobrogosz, W. J.** 1981. Metabolism, p. 388–390. *In* P. Gerhardt (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
4. **Fredericks, P. M., and L. A. Summers.** 1981. Synthesis and biological activity of aminomethylphosphonic acids related to the herbicide glyphosate. Z. Naturforsch. **36c**:242–245.
5. **Jacob, G. S., J. S. Schaefer, E. O. Stejskal, and R. A. McKay.** 1985. Solid-state NMR determination of glyphosate metabolism in a *Pseudomonas* sp. J. Biol. Chem. **260**:5899–5905.
6. **Jaworski, E. G.** 1972. Mode of action of N-phosphonomethylglycine: inhibition of aromatic amino acid biosynthesis. J. Agric. Food Chem. **20**:1195–1198.
7. **Moore, J. K., A. D. Larson, and H. D. Braymer.** 1983. Isolation of a *Pseudomonas* sp. which utilizes the phosphonate herbicide glyphosate. Appl. Environ. Microbiol. **46**:316–320.
8. **Nomura, N. S., and H. W. Hilton.** 1976. The absorption and degradation of glyphosate in five Hawaiian sugarcane soils. Weed Res. **17**:113–121.
9. **Rogers, S. G., L. A. Brand, S. B. Holder, E. S. Sharps, and M. J. Brackin.** 1983. Amplification of the *aroA* gene from *Escherichia coli* results in tolerance to the herbicide glyphosate. Appl. Environ. Microbiol. **46**:37–43.
10. **Rueppel, M. L., B. B. Brightwell, J. Schaefer, and J. T. Marvel.** 1977. Metabolism and degradation of glyphosate in soil and water. J. Agric. Food Chem. **25**:517–527.
11. **Sprankle, P., C. L. Sandberg, W. F. Meggitt, and D. Penner.** 1978. Separation of glyphosate and possible metabolites by thin-layer chromatography. Weed Sci. **26**:673–674.
12. **Steinrucken, H. C., and N. Amrhein.** 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl shikimic acid-3-phosphate synthase. Biochem. Biophys. Res. Commun. **94**:1207–1212.
13. **Talbot, H. W., L. M. Johnson, and D. M. Muccecke.** 1984. Glyphoste utilization by *Pseudomonas* sp. and *Alcaligenes* sp. isolated from environmental sources. Curr. Microbiol. **10**:255–260.
14. **Wood, H. G., and H. Gest.** 1957. Determination of formate. Methods Enzymol. **3**:285–292.
15. **Zweig, G., and J. Sherma.** 1972. Handbook of chromatography, p. 628. CRC Press, Boca Raton, Fla.