Phosphate Starvation Induces Uptake of Glyphosate by *Pseudomonas* sp. Strain PG2982

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Pseudomonas sp. strain PG2982 has the ability to use the phosphonate herbicide, glyphosate, as a sole phosphorus source (J. K. Moore, H. D. Braymer, and A. D. Larson, Appl. Environ. Microbiol. 46:316–320, 1983). Glyphosate uptake is maximal in the late log phase of growth and is induced by phosphate starvation. Uptake is inhibited by phosphate and arsenate, but not by the amino acids glycine and sarcosine. The K_m and V_{max} for glyphosate uptake were calculated to be 23 μM and 0.97 nmol/mg (dry weight) per min, respectively. A phosphate transport system with a broad substrate specificity may be responsible for glyphosate uptake.

Glyphosate (*N*-phosphonomethylglycine) is the active ingredient in the herbicide Roundup produced by the Monsanto Chemical Co., St. Louis, Mo. It is a potent inhibitor of 5-enolpyruvylshikimate-3-phosphate synthase, an enzyme involved in the synthesis of aromatic amino acids (5, 10). Moore et al. (4) previously described an organism (*Pseudomonas* sp. strain PG2982) which has the ability to utilize glyphosate, as well as several other phosphonate compounds (9), as a sole source of phosphorus. However, in the presence of P_i, glyphosate is not utilized and remains in the medium (J. K. Moore, M. S. thesis, Louisiana State University, Baton Rouge, 1983). We therefore investigated the uptake of glyphosate by PG2982 and the effect of phosphate on glyphosate uptake.

PG2982 was routinely grown and maintained on 1 mM glyphosate agar, which consisted of minimal medium plus 1 mM glyphosate (free acid form, 99.7% pure) as a phosphorus source. Minimal medium was composed of Dworkin-Foster salts without phosphate (1); gluconate (1% [wt/vol]) was added as a carbon source, and thiamine was added to 5 μg/ml. Phosphate, when added to media or to uptake assays, was in the form of monosodium orthophosphate (Na_2HPO_4). Arsenate, when added to uptake assays, was in the form of Na_2HAsPO_4.

For glyphosate uptake assays, broth cultures with 0.5 mM glyphosate, 0.5 mM phosphate, or 50 mM phosphate as the sole phosphorus source were used. Uptake assays were done by removing a portion of the culture, washing it once with minimal medium without phosphorus, and suspending it in minimal medium without phosphorus to an optical density at 600 nm of 1.0. [3-^{14}C]glyphosate was added to 1 mM or 100 μM, and at intervals, 100-μl samples of ^{14}C-labeled cells were filtered through 0.45-μm-pore-size Metricel membrane filters (Gelman Sciences Inc., Ann Arbor, Mich.). The filters were washed with 5 ml of minimal medium without phosphorus, dried, and placed in vials with 6 ml of Aqualyte Plus scintillation cocktail (J. T. Baker Chemicals, Deventer, The Netherlands). Radioactivity was measured with a LS6800 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.).

Breakdown of [3-^{14}C]glyphosate by PG2982 results in the evolution of $^{14}CO_2$ and the loss of radioactivity from the culture (8). To be sure that glyphosate was not broken down during uptake assays, 10-μl samples were taken before and

after the assays. These samples were not filtered but were counted directly. Counts before and after the assays were similar to counts taken from uninoculated controls, indicating that a significant breakdown of glyphosate had not taken place during the assays (data not shown).

Bacterial dry weight measurements were made by filtering cells through a 0.45-μm-pore-size membrane filter, washing the filters with 5 ml of minimal medium, drying these filters for 6 h at 60°C, and weighing the filters.

Glyphosate was assayed on a Beckman amino acid analyzer (model 120C) by the procedure of Moore et al. (4).

Initially, it was found that the rate of glyphosate uptake by PG2982 was quite variable during its growth cycle. Uptake by PG2982 was greatest at a point in the mid- to late log
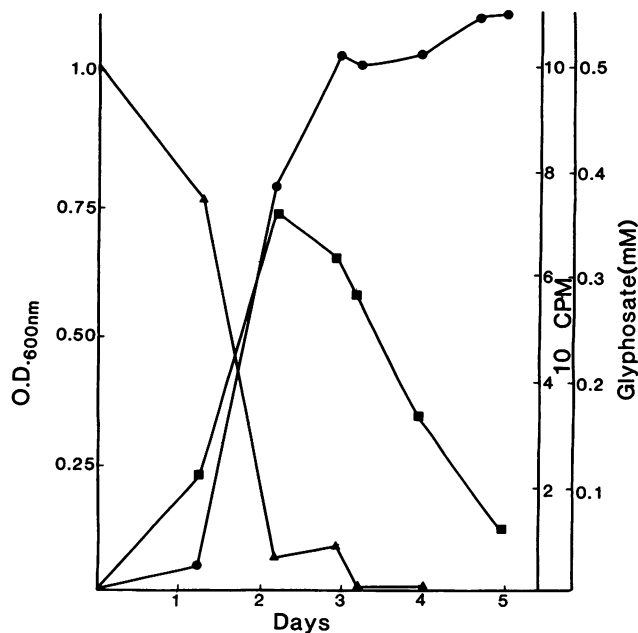

FIG. 1. Glyphosate uptake during growth of PG2982. Symbols: ●, growth of PG2982 as determined by measuring optical density at 600 nm (O.D.$_{600nm}$); ▲, glyphosate concentration in the culture medium; ■, counts per minute accumulated by 0.1 ml of cells during 15-min uptake assay with 1 mM [3-^{14}C]glyphosate.

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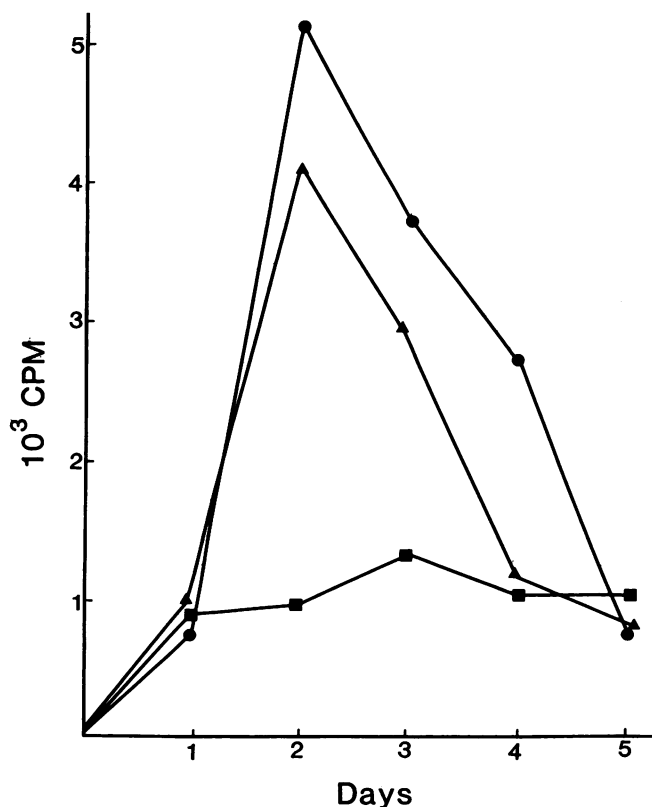
FIG. 2. Glyphosate uptake by cells grown in media containing 0.5 mM glyphosate (●), 0.5 mM Na₂HPO₄ (▲), or 50 mM Na₂HPO₄ (■) as the sole phosphorus source. Counts per minute accumulated by 0.1 ml of cells during 15-min uptake assay with 1 mM [3-^{14}C]glyphosate.

phase of growth (Fig. 1). This stage correlates with rapid disappearance of glyphosate from the growth medium. Before and after this point, the rate of glyphosate uptake decreased markedly.

Since the presence of phosphate inhibits the utilization of glyphosate (Moore, M.S. thesis), we investigated the uptake of glyphosate by cultures of PG2982 grown in minimal medium containing limiting (0.5 mM) and excess (50 mM) phosphate. PG2982 cells grown in minimal medium with 0.5 mM phosphate exhibited a glyphosate uptake pattern very similar to that of cells grown in minimal medium with 0.5 mM glyphosate (Fig. 2). However, when grown in minimal medium plus 50 mM phosphate, PG2982 did not take up significant amounts of glyphosate at any stage in its growth cycle. This suggests that uptake of glyphosate is regulated by the availability of phosphate. Also, glyphosate uptake by cells grown in minimal medium plus 0.5 mM phosphate was unaffected by the addition before the assay of chloramphenicol at 100 μg/ml (data not shown). Therefore, it seems that uptake is not induced by glyphosate itself, but rather by phosphate limitation.

Cells isolated at the stage in which glyphosate accumulation was at a maximum were used to investigate uptake further. It was found that phosphate inhibited uptake when it was added to cells during uptake assays. When phosphate was added at a concentration of 1 mM to uptake assays with 100 μM [3-^{14}C]glyphosate, uptake immediately stopped (Fig. 3). Arsenate, a phosphate analog, had the same effect. Since
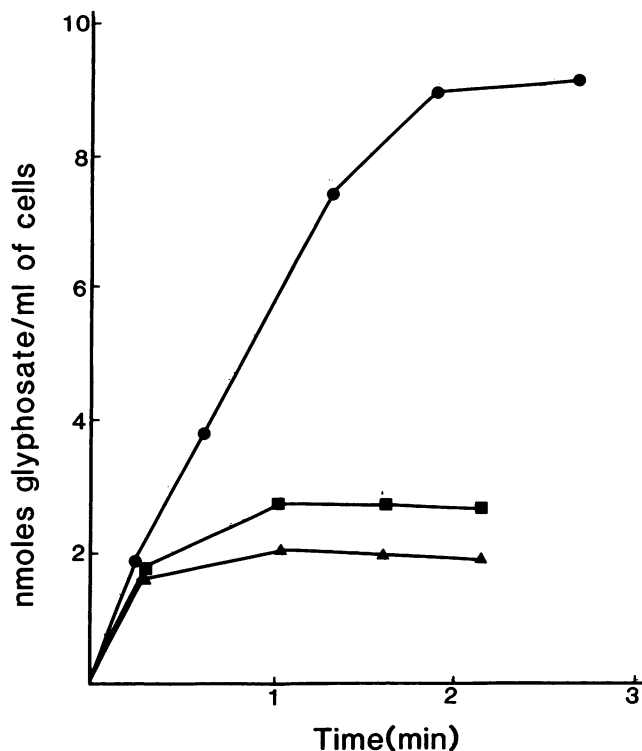

FIG. 3. Effect of phosphate and arsenate on glyphosate uptake, showing uptake of [3-^{14}C]glyphosate (initial concentration, 0.1 mM) by PG2982 (●). After 30 s, Na₂HPO₄ (■) or Na₂HAsO₄ (▲) was added to 1 mM.

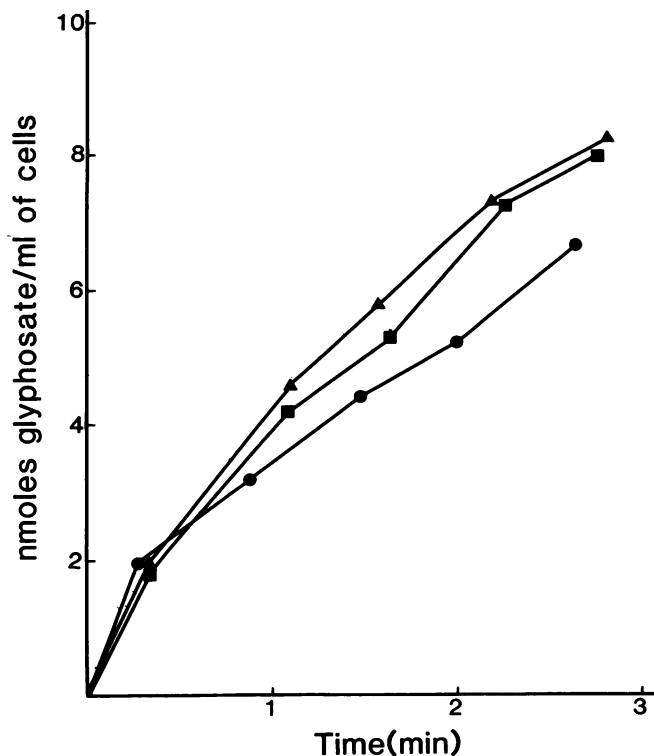FIG. 4. Effect of glycine and sarcosine on glyphosate uptake, showing uptake of [3-^{14}C]glyphosate (initial concentration, 0.1 mM) by PG2982 (●). After 30 s, glycine (▲) or sarcosine (■) was added to 1 mM.
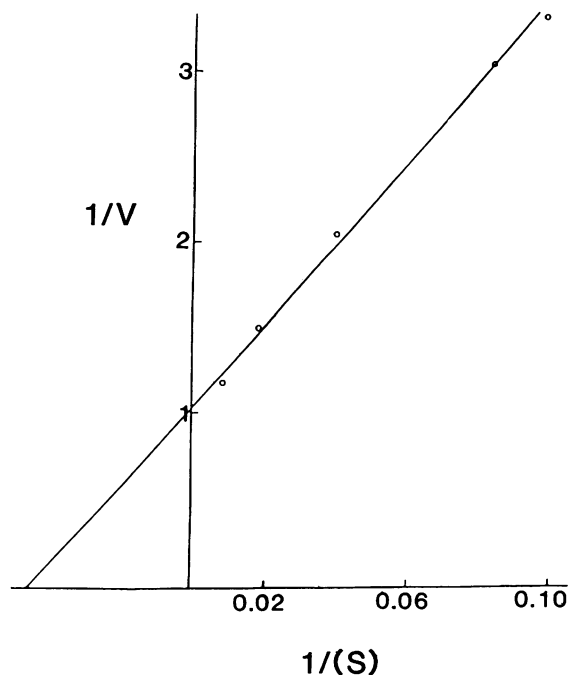
1/(S)

FIG. 5. Kinetic parameters of glyphosate uptake by PG2982. V, Nanomoles of glyphosate per milligram (dry weight) per minute; S, nanomoles per milliliter.

a portion of the glyphosate molecule is structurally similar to that of the amino acid glycine, we also investigated the possibility that it is taken up by a glycine transport system. However, when 1 mM glycine was added to uptake assays containing 100 μM [3-^{14}C]glyphosate, no inhibition of uptake was seen (Fig. 4). Similarly, sarcosine, which is also structurally related to glyphosate, had no effect on glyphosate uptake.

A Lineweaver-Burk plot of glyphosate uptake was made by measuring initial uptake rates at various glyphosate concentrations between 1 and 100 μM. The K_m was determined to be 23 μM, and the V_{max} was 0.97 nmol/mg (dry weight) per min (Fig. 5).

We are not aware of any previous studies of glyphosate uptake by bacteria. However, uptake of aminophosphonic acids other than glyphosate has been studied with some bacteria. The glyphosate uptake system of PG2982 seems to differ from the systems for other aminophosphonic acids reported for these bacteria.

Uptake of aminophosphonic acids analogous to glutamic acid, aspartic acid, alanine, and valine has been studied in *Streptococcus faecalis* and *Lactobacillus plantarum* (2). These aminophosphonic acids were found to be accumulated by amino acid transport systems. However, cultures of these bacteria were grown and assayed in high concentrations of phosphate.

Rosenberg and LaNauze (7) have reported the utilization of aminoethylphosphonate as the sole phosphorus source by a strain of *Bacillus cereus*. However, aminoethylphosphonate was not used in the presence of phosphate, and induction of the transport system was suppressed by phosphate. In the absence of phosphate, the transport system was induced by the substrate aminoethylphosphonate.

The glyphosate uptake system of PG2982 seems to be unlike either of these uptake systems. Since neither of the structurally related amino acids glycine or sarcosine was able to compete with glyphosate in uptake assays, it is

unlikely that an amino acid transport system is responsible for the high level of glyphosate uptake found in PG2982. As in the case of *B. cereus*, excess phosphate does inhibit induction of glyphosate transport. However, glyphosate itself is not required for induction. In this case, phosphate starvation alone is sufficient for induction.

In *Escherichia coli*, many genes are known to be induced by phosphate starvation (12). Several proteins are involved in the uptake of phosphate and phosphorylated compounds from the medium (11). Among these are proteins involved in a high-affinity phosphate transport system which is specific for P_i (6). An analogous high-affinity system is known to operate in *Pseudomonas aeruginosa*, but it has a wider substrate specificity (3). In *P. aeruginosa*, arsenate can compete with phosphate through the high-affinity system, as can methylphosphonate and other aminophosphonic acids. We feel that a similar system may operate in PG2982 to take up glyphosate. This system apparently has a broad specificity, since it is responsible for the uptake of phosphate, arsenate, and glyphosate. Like *P. aeruginosa*, PG2982 may have developed a transport system with a high affinity for P_i and a lower affinity for various phosphorus-containing compounds, including glyphosate.

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