Metabolism of Phosphonoacetate as the Sole Carbon and Phosphorus Source by an Environmental Bacterial Isolate

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A gram-negative bacterium isolated from activated sludge was able to utilize up to 25 mM phosphonoacetate as the sole carbon and phosphorus source, with simultaneous excretion of virtually equimolar levels of phosphate. 2-Aminoethylphosphonate was similarly utilized with equivalent growth rates and cellular yields, while 3-aminopropyl-, 4-aminobuty1-, methyl-, ethyl-, and phenylphosphonates served only as phosphorus sources.

A wide range of organophosphonates (compounds containing a stable carbon-to-phosphorus [C—P] bond) can be metabolized by bacteria as the sole phosphorus source (10). At least two enzymes have been implicated in C—P bond cleavage. Phosphonatase (phosphonoacetaldehyde hydrolase; EC 3.11.1.1) has been shown to be specific to the activated C—P bond of phosphonoacetate dehydrogenase; C—P lyase appears to have broad substrate specificity and to carry out a direct C—P bond cleavage, although no activity has been obtained in cell extracts (13). Evidence for a third C—P bond cleavage enzyme, termed C-P hydrolase, has been presented (8), but no organic product could be detected.

Two organisms have been reported to utilize organophosphonates as the sole carbon source. *Pseudomonas putida* metabolized phosphonoacetaldehyde via acetaldehyde while *Agrobacterium radiobacter* degraded the herbicide glyphosate via carbon—nitrogen bond cleavage with utilization of the resulting glyoxylate as the sole carbon source (7). However, no organism has been reported that will utilize an organophosphonate as a carbon source by direct cleavage of the C—P bond by C-P lyase. This may be explained by the regulation of both phosphonate uptake and C-P lyase activity by phosphate (9); both transport and catabolism of an organophosphonate would thus be repressed and/or inhibited by excess phosphate released during utilization of its organic moiety.

We report the isolation of an environmental microorganism capable of utilizing an organophosphonate, other than phosphonoacetaldehyde and glyphosate, as a sole carbon and phosphorus source. The gram-negative bacterial isolate, designated 23F, could use the synthetic phosphonate antiviral antibiotic carboxymethylphosphonate (phosphonoacetate) (1) as a sole carbon source or as a carbon and phosphorus source with essentially quantitative extracellular release of phosphonate phosphorus as orthophosphate.

The organophosphonates listed in Table 1 were used in this study. The basal culture medium contained the following: Tris HCl, 6.0 g/liter; NH₄Cl, 5.0 g/liter; CaCl₂ - 2H₂O, 0.08 g/liter; MgSO₄ - 7H₂O, 0.16 g/liter; vitamin solution, 1.0 ml/liter (5); trace element solution, 1.0 ml/liter (5); phosphate-free yeast extract, 0.05 g/liter (pH 7.2). The yeast extract was prepared by the addition of MgO (10 g) to 1 liter of yeast extract solution (200 g liter⁻¹), the mixture was agitated (4 h at 5°C) and centrifuged (5,000 rpm for 20 min), and the supernatant was removed and filter sterilized (14).

Filter-sterilized phosphonoacetate (20 mM) was routinely used as the sole carbon and phosphorus source.

Enrichment culture was carried out by using a 0.5% (vol/vol) inoculum from an activated-sludge plant treating laundry wastes at Dunmurry, Northern Ireland. Flasks (250 ml) containing 50 ml of medium were incubated at 29°C on a reciprocal shaker at 80 rpm.

Growth of cultures was measured as A₅₅₀, while phosphate levels in culture supernatants were assayed by the method of Fiske and SubbaRow (4). The presence of acetate and acetaldehyde in culture supernatants was assayed by using enzyme-based Acetate and Acetaldehyde Test Kits (Boehringer Mannheim); lower limits of detection were 10 and 5 mg liter⁻¹, respectively.

After eight serial transfers, a bacterial isolate, designated 23F, capable of growth on 20 mM phosphonoacetate as the sole carbon and phosphorus source was purified by plating on medium solidified with 1.2% Bacto Agar (Difco). The isolate was gram negative, nonpigmented, and oxidase and catalase positive.

The ability of isolate 23F to utilize a range of organophosphonates as the sole carbon and phosphorus source, or as the sole phosphorus source with glucose (5 g liter⁻¹) as the carbon source, is shown in Table 1; its growth on 10 mM phosphonoacetate as the sole carbon and phosphorus source is shown in Fig. 1. The inocula (0.5% [vol/vol]) used in these experiments consisted of late-log-phase cells pregrown in the same medium and contained approximately 9 μmol of orthophosphate ml⁻¹; an identical result was obtained if a washed inoculum in which phosphate levels were reduced to 35 nmol ml⁻¹ was used.

Growth of isolate 23F on phosphonoacetate (Fig. 1) or 2-aminoethylphosphonate as the sole carbon and phosphorus source was accompanied by a concomitant release of phosphate. Uninoculated control experiments confirmed that neither phosphate released orthophosphate spontaneously during incubation. Extracellular accumulation of >95% phosphonate phosphorus as orthophosphate was found in culture supernatants at phosphonoacetate concentrations up to 25 mM. This result was anticipated since a carbon/phosphorus molar ratio in excess of 200:1 is necessary to effect phosphorus limitation of bacterial growth (2).

Decreasing growth rates were observed when isolate 23F was grown at higher phosphonoacetate levels, and no growth occurred at concentrations greater than 25 mM. However, final cell yields of the organism grown on phosphonoacetate as the sole C and P source were directly proportional to the phosphonoacetate concentration within the range of 0 to 25

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mM (Fig. 2). Neither acetate nor acetaldehyde were detected when the supernatants of cultures growing on 20 mM phosphonoacetate as the sole phosphorus source were assayed for intermediates.

The findings reported are surprising in light of extensive evidence derived from diverse bacterial groups of the stringent control of organophosphonate uptake and metabolism by inorganic phosphate, whether the enzyme involved is phosphonatase (11) or C-P lyase (9, 13). Our failure to detect either acetaldehyde or acetate (potential products, respectively, of the action of phosphonatase and C-P lyase [12]) in culture supernatants, leaves the route of phosphonoacetate metabolism by isolate 23F still uncertain. Future demonstration of enzyme activity in cell extracts or permeabilized cells should allow a rapid elucidation of the degradation pathway. Since phosphonoacetate contains a carboxyl group adjacent to its C—P bond, it would be of particular interest to determine if the enzymatic mechanism of metabolism proceeds via a phosphonatase or a C-P lyase-like reaction. Although we have isolated some 20 environmental bacterial strains capable of hydrolysis of 2-aminoethylphosphonate and its utilization as the sole C and P source via phosphonatase, none of these could similarly utilize phosphonoacetate. In addition, isolate 23F is able to metabolize a number of unsubstituted phosphonates as the sole phosphorus source (Table 1). A further unusual feature of phosphonoacetate metabolism by isolate 23F is the fact that even phosphorus-depleted cells are capable of its utilization as the sole carbon and phosphorus source; this would argue against the postulated existence in this instance of a tightly-coupled phosphonoacetate-phosphate antiporter (2), which would necessitate strictly compartmented and effectively independent metabolism of carbon and phosphorus (6).

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