Volume 236, number 1, 135–138 FEB 06181 August 1988

# Carbon-phosphorus lyase activity in permeabilized cells of *Arthrobacter* sp. GLP-1

Rüdiger Pipke* and Nikolaus Amrhein

*Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität Bochum, D-4630 Bochum, FRG*

Received 20 June 1988

*Arthrobacter* sp. GLP-1 is capable of using the phosphonate herbicide glyphosate (*N*-[phosphonomethyl]glycine) as its sole source of phosphorus. The formation of sarcosine from glyphosate by a carbon-phosphorus (C-P) lyase activity could be demonstrated in vivo, as well as in situ in cells permeabilized by 2% dimethylsulfoxide, when sarcosine oxidase was inhibited by acetate. The apparent $K_m$ of the C-P lyase for glyphosate was found to be 61 $\mu$M, and a $V$ of 2.2 pmol · $s^{-1}$ · $mg^{-1}$ soluble protein was determined. Uptake of glyphosate into the cells and C-P lyase activity represent distinct processes.

Carbon-phosphorus lyase; Glyphosate; Herbicide; Phosphonate degradation

## 1. INTRODUCTION

The bacterial metabolism of organophosphonates has found much interest because of the natural occurrence of such compounds as well as the increasing release of numerous phosphonate-containing xenobiotics, such as the herbicide glyphosate (*N*-[phosphonomethyl]glycine), into the environment [1]. The biochemistry of the cleavage of the carbon to phosphorus bond is poorly understood because, to date, no cell-free preparations of carbon-phosphorus (C-P) lyase activity have been obtained [2–4]. A single apparent exception is the phosphonoacetaldehyde hydrolase (phosphonatase, EC 3.11.1.1) of *Bacillus cereus* which is involved in the degradation of the naturally occurring 2-aminoethylphosphonate [5]. This enzyme is highly specific for phosphonoacetaldehyde, the transamination product of 2-aminoethylphosphonate. Whereas the hydrolytic cleavage of phosphonoacetaldehyde occurs via an intermediate imine formation [6], the cleavage of the unactivated C-P bond of alkylphosphonates by C-P lyase(s) represents a functionally distinct reaction [7,8]. Several bacterial strains have been shown to possess C-P lyase activity which enables them to use a wide range of alkylphosphonates as their sole source of phosphorus (see [1] for references). It has been proposed that the C-P lyase catalysed reaction involves radical intermediates [7,8]. Evidence was recently presented that the initial step in the degradation of the phosphonate herbicide glyphosate by *Arthrobacter* sp. GLP-1 and *Pseudomonas* sp. PG 2982 is the formation of sarcosine (*N*-methylglycine) [3,9,10]. While it was not possible to demonstrate glyphosate-degrading activity in a cell-free system [3,9,11], we report here an in situ assay of such activity in permeabilized cells of *Arthrobacter* sp. GLP-1.

*Correspondence (present) address:* N. Amrhein, Institut für Pflanzenwissenschaften, Biochemie und Physiologie der Pflanzen, ETH-Zürich, Sonneggstr. 5, CH-8092 Zürich, Switzerland

* *Present address:* Gesellschaft für Biotechnologische Forschung, Abteilung Mikrobiologie, Mascheroder Weg 1, D-3300 Braunschweig, FRG

## 2. MATERIALS AND METHODS

Isolation and cultivation of *Arthrobacter* GLP-1 as well as the uptake experiments have been described [3,12]. The reaction

*Published by Elsevier Science Publishers B.V. (Biomedical Division)*
00145793/88/$3.50 

mixture for the conversion of glyphosate to sarcosine in vivo contained in a total volume of 40 $\mu$l: 50 mM Tris-HCl, pH 7.2; 10 mM sodium acetate; 50 $\mu$M [3-$^{14}$C]glyphosate (180 MBq/mmol) and 5–10 $\times$ $10^9$ cells $\cdot$ ml$^{-1}$ in the logarithmic growth phase ($A_{650}$ = 1.0). After 30 min at 30°C, the reaction was interrupted by centrifugation (1 min, 15000 $\times$ $g$), and 5 $\mu$l aliquots of the supernatant subjected to thin-layer chromatography. To assess C-P lyase activity in situ, 2% dimethylsulfoxide (v/v) was added to the reaction mixture. Protein concentrations were determined after sonication and centrifugation of the bacteria [13]. Separation of glyphosate from sarcosine was readily achieved by thin-layer chromatography on cellulose sheets using isobutyric acid/water/1-propanol/conc. $NH_4OH$/2-propanol/1-butanol (500:95:70:20:15:15, by vol.) containing 0.24 g of disodium EDTA/l as solvent [14]. Radioactivity on the chromatograms was calculated using an Isomess IM 3000 radioscanner.

## 3. RESULTS

The failure to demonstrate C-P lyase (glyphosate-degrading) activity in cell-free preparations of *Arthrobacter* sp. GLP-1 [3] led us to investigate alternative procedures for assessing the activity of this enzyme. The in vivo formation of sarcosine from glyphosate had previously been corroborated by thin-layer chromatography and enzymatic analysis of the product [3], as well as by automated amino acid analysis (unpublished). To prevent breakdown of sarcosine by sarcosine oxidase in the intact cells, sodium acetate was added to the incubation mixture to inhibit this enzyme [15]. In the presence of sodium acetate, the bacteria released easily detectable amounts of labelled sarcosine from [3-$^{14}$C]glyphosate (fig.1), and it became possible to estimate the in vivo C-P lyase activity. Attempts to improve the assay by permeabilization of the cells with either ethanol/toluene [16] or cetyltrimethylammonium bromide [17] resulted in the complete loss of sarcosine formation. However, the addition of 2% dimethylsulfoxide significantly enhanced the formation and release of sarcosine from glyphosate (fig.1). Subsequently, the C-P lyase activity in situ was further investigated under these optimized conditions (10 mM sodium acetate, 2% dimethylsulfoxide). The formation of sarcosine from 50 $\mu$M glyphosate was linear over an interval of 60 min and up to protein concentrations of 5 mg $\cdot$ ml$^{-1}$ (not shown). The C-P lyase activity exhibited Michaelis-Menten kinetics, and an apparent $K_m$ value of 61 $\mu$M for glyphosate and a $V$ of

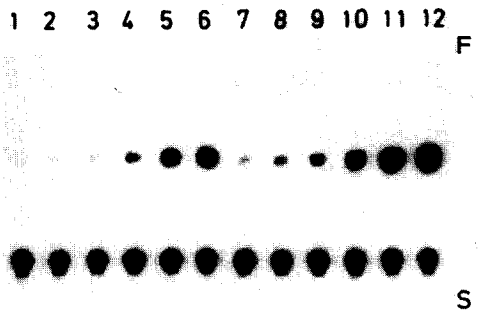


Fig.1. Glyphosate degradation by *Arthrobacter* sp. GLP-1. Cells in the logarithmic phase of growth in the presence of 1 mM glyphosate as the source of phosphorus were washed and then incubated with 50 $\mu$M [3-$^{14}$C]glyphosate. At 20 min intervals, aliquots of the supernatants were subjected to thin-layer chromatography and the chromatograms autoradiographed. S, start; F, front; $R_f$ glyphosate, 0.15; $R_f$ sarcosine, 0.50. Lanes: 1–3, 20, 40 and 60 min, glyphosate only; 4–6, as above, plus 10 mM sodium acetate; 7–9, as lanes 1–3, plus 2% dimethylsulfoxide; 10–12, as above, plus 10 mM sodium acetate.

2.2 pmol $\cdot$ s$^{-1}$ $\cdot$ mg$^{-1}$ were determined from a Lineweaver-Burk plot (not shown).

We had previously shown that *Arthrobacter* sp. GLP-1 utilizes glyphosate only in the absence of orthophosphate from the growth medium [3]. Furthermore, since orthophosphate inhibits, as well as represses, glyphosate uptake by the bacteria [12], it could, previously, not clearly be established whether uptake and breakdown of glyphosate

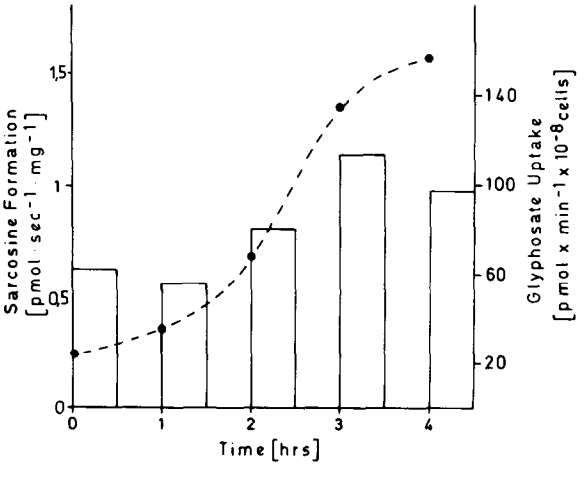


Fig.2. Rate of glyphosate uptake and C-P lyase activity in cells of *Arthrobacter* sp. GLP-1 after transfer from medium with 1 mM glyphosate to medium without a source of phosphorus. (Bars) Sarcosine formation; (●--●) glyphosate uptake.

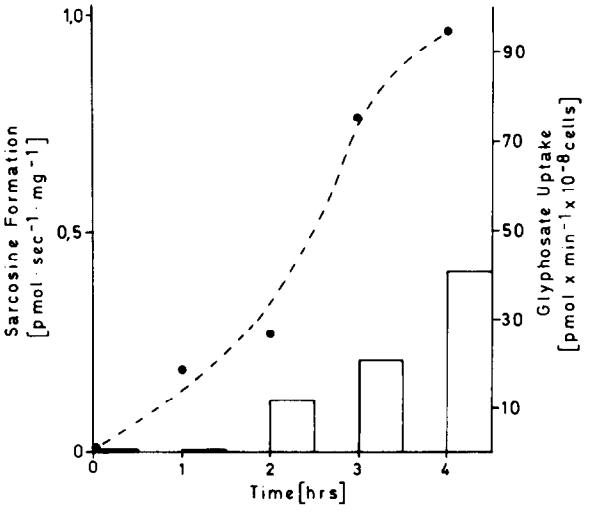


Fig.3. Rate of glyphosate uptake and C-P lyase activity in cells of *Arthrobacter* sp. GLP-1 after transfer from medium with 1 mM orthophosphate to medium without a source of phosphorus. (Bars) Sarcosine formation; (●----●) glyphosate uptake.

represent distinct processes. With the advent of the in situ assay of the C-P lyase, this question was now reinvestigated. Rates of uptake and C-P lyase activities were monitored in cells which had grown in medium containing glyphosate as the sole source of phosphorus and were subsequently transferred to medium without any source of phosphorus (fig.2). 4 h after withdrawal of glyphosate, the rate of glyphosate uptake had increased 8-fold, while the C-P lyase activity was elevated only slightly.

When bacteria were grown in the presence of 1 mM orthophosphate as their sole source of phosphorus and then transferred to medium without any source of phosphorus, it became apparent that phosphate suppressed both glyphosate uptake and C-P lyase activities (fig.3). Furthermore, upon withdrawal of the source of phosphorus, both activities were induced, exhibiting, however, different induction kinetics (fig.3). It can therefore be concluded that glyphosate uptake and breakdown are separate events.

## 4. DISCUSSION

As an approximation to a system containing a cell-free C-P lyase activity, we have shown here that permeabilized cells of *Arthrobacter* sp. GLP-1 degrade glyphosate to sarcosine which is released into the incubation mixture provided that further sarcosine metabolism is inhibited by acetate (fig.1). The establishment of an in situ assay for the C-P lyase (glyphosate-degrading) activity allowed us to investigate the metabolic regulation of glyphosate uptake and breakdown. While it is evident that both processes are subject to strict control by the supply of phosphorus to the cells (figs 2 and 3), they are apparently distinct from each other.

Recently, genetic approaches have been chosen to identify the C-P lyase(s) of *Escherichia coli* which is capable of using a variety of alkylphosphonates, but not glyphosate, as its sole source of phosphorus. In this bacterium, the C-P lyase activity is regulated as a part of the phosphate regulon, and by transposon mutagenesis the *psi* D locus was shown to be involved in methylphosphonate degradation [18]. Efforts are currently directed toward the identification of the *psi* D gene product. In an additional effort, a mutant of *E. coli* deficient in organophosphonate degradation was complemented with a cosmid library of wild type DNA, and the complementing DNA was characterized by restriction analysis [19]. A combination of biochemical and genetic approaches should ultimately allow the identification of the elusive C-P lyase(s).

*Acknowledgement:* Support of this work by a grant-in-aid from Monsanto Agricultural Products Co., St. Louis, MO, is gratefully acknowledged.

## REFERENCES

[1] Hilderbrand, R.C. (1983) The Role of Phosphonates in Living Systems, CRC Press, Boca Raton, FL.
[2] Wackett, L.P., Shames, S.L., Venditti, C.P. and Walsh, C.T. (1987) J. Bacteriol. 169, 710–717.
[3] Pipke, R., Amrhein, N., Jacob, G.S., Schaefer, J. and Kishore, G.M. (1987) Eur. J. Biochem. 165, 267–273.
[4] Frost, J.W., Loo, S., Cordeiro, M.L. and Li, D. (1987) J. Am. Chem. Soc. 109, 2166–2171.
[5] La Nauze, J.M., Rosenberg, H. and Shaw, D.C. (1970) Biochim. Biophys. Acta 212, 332–350.
[6] La Nauze, J.M., Coggins, J.R. and Dixon, H.B.F. (1977) Biochem. J. 165, 409–411.
[7] Cordeiro, M.L., Pompliano, D.L. and Frost, J.W. (1986) J. Am. Chem. Soc. 108, 332–334.
[8] Shames, S.L., Wackett, L.P., LaBarge, M.S., Kuczkowski, R.L. and Walsh, C.T. (1987) Bioorg. Chem. 15, 366–373.

[9] Shinabarger, D.L. and Braymer, H.O. (1986) J. Bacteriol. 168, 702-707.
[10] Kishore, G.M. and Jacobs, G.S. (1987) J. Biol. Chem. 262, 12164-12168.
[11] Moore, J.K., Braymer, H.D. and Larson, A.D. (1983) Appl. Environ. Microbiol. 46, 316-320.
[12] Pipke, R., Schulz, A. and Amrhein, N. (1987) Appl. Environ. Microbiol. 53, 974-978.
[13] Steinrücken, H.C. and Amrhein, N. (1984) Eur. J. Biochem. 143, 341-349.
[14] Rueppel, M.L., Brightwell, B.B., Schaefer, J. and Marvel, J.T. (1977) J. Agric. Food Chem. 25, 517-528.
[15] Barman, T.E. (1969) Enzyme Handbook, vol. 1, p. 193, Springer, New York.
[16] Kornberg, H.L. and Reeves, R.E. (1972) Biochem. J. 126, 1241-1243.
[17] White, P.J., Kelly, B., Suffling, A. and Work, E. (1964) Biochem. J. 91, 600-610.
[18] Wackett, L.P., Wanner, B.L., Venditti, C.P. and Walsh, C.T. (1987) J. Bacteriol. 169, 1753-1756.
[19] Loo, S.H., Peters, N.K. and Frost, J.W. (1987) Biochem. Biophys. Res. Commun. 148, 148-152.