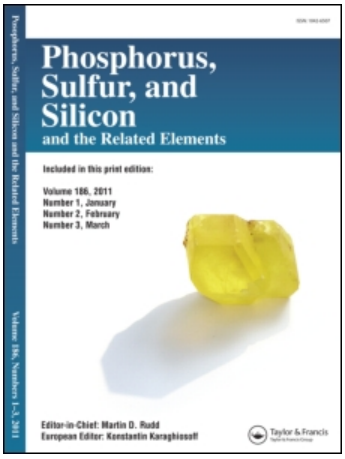


Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

The Degradation Effects of a *Pseudomonas* Hydrolase OPHC2 to Organophosphorus Insecticides

Jin-Fang Wang[a]; Ming-Hui Gao[a]; Ning-Feng Wu[b]; Can-Ping Pan[a]
[a] College of Science, China Agricultural University, Beijing, China [b] Chinese Academy of Agricultural Sciences, Biotechnology Research Institute, Beijing, China

To cite this Article Wang, Jin-Fang , Gao, Ming-Hui , Wu, Ning-Feng and Pan, Can-Ping(2008) 'The Degradation Effects of a *Pseudomonas* Hydrolase OPHC2 to Organophosphorus Insecticides', Phosphorus, Sulfur, and Silicon and the Related Elements, 183: 2, 804 — 810
To link to this Article: DOI: 10.1080/10426500701808234
URL: http://dx.doi.org/10.1080/10426500701808234

Phosphorus, Sulfur, and Silicon, 183:804–810, 2008

ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426500701808234

Taylor & Francis
Taylor & Francis Group

The Degradation Effects of a *Pseudomonas* Hydrolase OPHC2 to Organophosphorus Insecticides

Jin-Fang Wang,[1] Ming-Hui Gao,[1] Ning-Feng Wu,[2] and Can-Ping Pan[1]

[1]College of Science, China Agricultural University, Beijing 100094, China

[2]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China

The degradation effects of OPHC2 to 9 organophosphates and other pesticides were studied. The degrading reaction was carried out for 10 minutes under 37°C in 50 mmol pH 8.0 Tris-HCl buffer. Organophosphates and sulfonylurea herbicides were detected by HPLC-UV and/or HPLC/MS. It's shown that degradation rates of 70%–100% were achieved for most of the organophosphates after 24 h (chlorpyrifos 100%, phoxim 100%, fenitrothion 99.87%, isocarbophos 100%, dimethoate 78.55%, methamidophos 71.55%, methidathion 87.93%). Pyrethroids were detected by GC-ECD after extracted by hexane. The degrading amounts of 5 pyrethroids were all above 50% after 3 hours (bifenthrin 78.7%, lambda-cyhalothrin 84.6%, permethrin 69.1%, beta-cypermethrin 51.5%, fenvalerate 72.2%). What is interesting, the degrading amount of malathion in its single standard solution was 94.31% while it did not degrade at all in the mixture standard even under superfluous enzyme solution. OPHC2 showed no effect to the sulfonylurea herbicides.

Keywords Enzymatic degradation; OPHC2; organophosphates; *Pseudomonas* hydrolase; pyrethroids; sulfonylurea herbicides

INTRODUCTION

From the 20th century, pesticides such as pyrethroids, organophosphates, and sulfonylurea herbicides are commonly used throughout the world and have made a great contribution to agriculture. However, pesticides also bring us some problems like environment pollution and health threatener.[1–3] In the past ten years, people have focused on finding some enzymes to degrade pesticides.[4–10] Recently, a bacterium

This research was supported by China National Basic Research and Development Program-2002CB410805.

Address correspondence to Canping Pan, College of Science, China Agricultural University, Beijing 100094, China. E-mail: canpingp@cau.edu.cn

strain C2-1 with the capability of degrading organophosphorus, identified as *Pseudomonas pseudoalcaligenes*, was isolated from OP-treated soil. The new hydrolase OPHC2 from this bacterium was purified and characterized, using PAGE gel to obtain three main protein bands and the objective one was in the middle. A single band of the purified OPHC2 was gained by the SDS-PAGE gel with about 36 kD molecular weight.[11] The sequence of OPHC2 has been deposited in GenBank with the accession number AJ605330.

In this article, the degrading effects of OPHC2 to 9 organophosphates (chlorpyriphos, isocarbophos, malathion, dimethoate, methamidophos, methidathion, fenitrothion, phoxim, and parathion), 5 pyrethroids (bifenthrin, lambda-cyhalothrin, permethrin, beta-cypermethrin, fenvalerate), and 10 sulfonylurea herbicides (nicosulfuron, thifensulfuron-methyl, metsulfuron-methyl, sulfometuron-methyl, chlorsulfuron, ethametsulfuron-methyl, tribenuron, bensulfuron-methyl, pyrazosulfuron-ethyl, chlorimuron-ethyl) were studied.

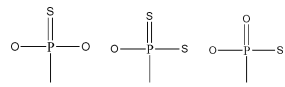
RESULTS AND DISCUSSION

Determination of the Enzymatic Degrading Reaction Conditions

The optimum degrading temperature and pH of OPHC2 are different according to specified pesticides, however, it has a good stability and a broad pH range, it is active between 20°C and 80°C, from pH 6 to 11.[12] The experiments in this article were taken under 37°C and pH 8.0 Tris-HCl buffer.

The Degrading Effects of OPHC2 to Organophosphates

All the organophosphates in standard solution without enzyme addition showed no degradation within 48 h. However, as to those added by enzyme, the degrading amounts were from about 70% to 100% for single organphosphorus standards after 24 h, chlorpyrifos (100%), phoxim (100%), fenitrothion (99.87%), isocarbophos (100%), dimethoate (78.55%), malathion (94.31%), methidathion (87.93%), and methamidophos (71.55%), respectively. Apparently, The former four pesticides were almost totally hydrolyzed. This may be due to their special structures. The former four pesticides possess similar structure with **a**, while the latter four pesticides possess similar structure with **b** or **c** (methamidophos). For the mixed standard composed of chlorpyriphos, isocarbophos, malathion, methamidophos, methidathion, fenitrothion, and parathion, the degrading curves of 7 Ops was showed in Figure 1. We can see that most organophosphates are easily to be hydrolyzed



a b c

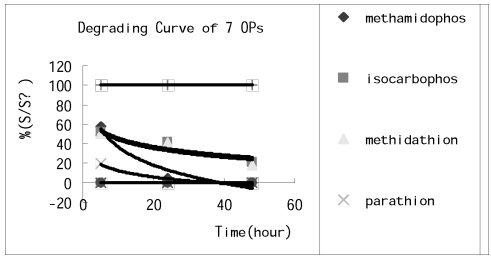


FIGURE 1 Degrading curves of 7 OPs in mixture standard solution. S: the peak area of a compound in the mixture standard solution with enzyme addition after a certain degrading time. S_0: the peak area of a compound in the mixture standard solution without enzyme addition.

by OPHC2 except malathion. So malathion did not show any degradation in the mixture standard while it can be easily degraded in its single standard. The factors leading to this phenomenon are not clear so far, maybe because steric hindrance in the enzyme reaction plays an inhibitory effect or there is interaction between the 7 pesticides and OPHC.

The Degrading Effects of OPHC2 to Pyrethroids

Results showed that the degrading effect of OPHC2 to pyrethroids differed from the concentration of enzyme solution, and the result of adding 50 μL enzyme solution is the best. As shown in Figure 2, (a) is 1 mg/L mixed standard solution without enzyme, (b) is the degradation chromatography after 3 h reacted with 50 μL enzyme solution for 10 min. The degrading amounts of 5 pyrethroids by OPHC2 are all above 50% after 3 h, bifenthrin 78.7%, lambda-cyhalothrin 84.6%, permethrin 69.1%, beta-cypermethrin 51.5%, fenvalerate 72.2%.

Mechanism of Enzymatic Degrading Reaction

As for sulfonylurea herbicides, there was no degrading effect of OPHC2 to them even after 48 h. So we can conclude that OPHC2 has a good degrading effect to pyrethroids and organophosphates, like other

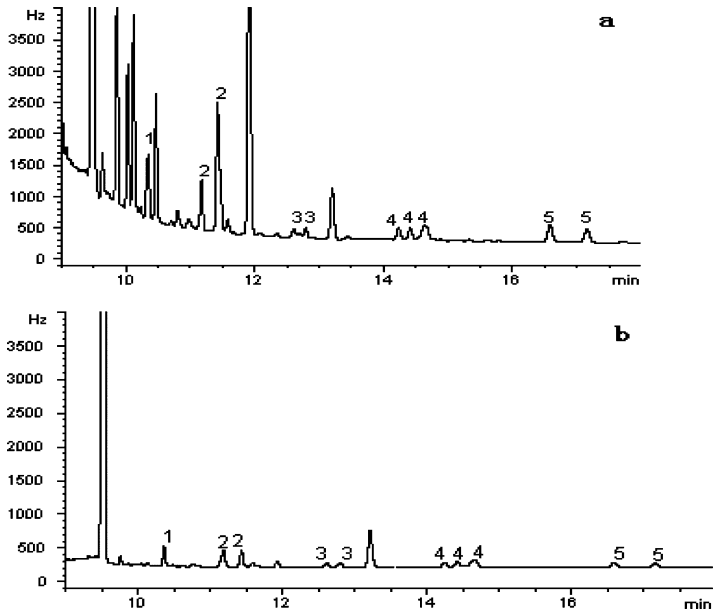


FIGURE 2 Typical LC-UV chromatogram of 1 mg/L mixed standard solution of 5 pyrethroids after 3 hours degradation reaction (b) and compared chromatography without enzyme (a). 1-bifenthrin, 2-lambda-cyhalothrin, 3-permethrin, 4-beta-cypermethrin, 5-fenvalerate.

hydrolases reported by some articles,[13–15] but it has no effect to sulfonylurea herbicides. Hence, it indicates that OPHC2 can hydrolase ester bonds but has no effect to other functional groups.

CONCLUSION

As OPHC2 can degrade many organophosphates and pyrethroids, it is useful and important to study about immobilizing this *Pseudomonas* hydrolase or preparing corresponding biosensor to deal with the correlative pollution to the environment, especially to soil or fields that are near chemical factories.

EXPERIMENTAL

Chemicals and Reagents

The pesticides investigated were 9 organophosphates (chlorpyriphos, isocarbophos, malathion, dimethoate, methamidophos, methidathion, fenitrothion, phoxim, and parathion.), 5 pyrethroids (bifenthrin,

lambda-cyhalothrin, permethrin, beta-cypermethrin, fenvalerate) and 10 sulfonylurea herbicides (nicosulfuron, thifensulfuron-methyl, metsulfuron-methyl, sulfometuron-methyl, chlorsulfuron, ethametsulfuron-methyl, tribenuron, bensulfuron-methyl, pyrazosulfuron-ethyl, chlorimuron-ethyl). They were purchased from Institute for Control of Agrichemicals Ministry of Agriculture (China), and the purities of them are all above 98%.

Acetone, n-hexane, methanol, acetonitrile, HCl (37%), of special grading for the pesticide residue analysis, were purchased from Burdick & Jackson Company (USA). Water was gained from Milli-Q water purification system (0.22 μm) from Millipore. Tris(hydroxymethyl) aminomethane (99.9%) was purchased from Beijing Chemical Reagents Company (China).

Stock solutions of each pyrethroid of 1000 mg/L were prepared in acetone. A standard mixture of 10 mg/L of each pyrethroid in acetone was prepared from the stock solutions. As to organophosphates and sulfonylurea herbicides, stock solutions (1000 mg/L) of individual pesticide standards were prepared by dissolving 0.010 g of the pesticide in 10 mL acetonitrile. The mixed standard solutions of organophosphates and sulfonylurea herbicides (100 mg/L of each pesticide) were prepared by an appropriate dilution with acetonitrile individually. All the solutions were stored in a freezer at 4°C.

Tris-HCl buffer (pH 8.0). Mixed 50 mL 0.1 mol/L Tris-alkali with 29.2 mL 0.1 mol/L HCl, then diluted to 100 mL with water, making 50 mmol/L pH 8.0 Tris-HCl buffer.

Enzyme solution. Weighed 25 mg hydrolase OPHC2 into 2 mL centrifugal tube, added 500 μL Tris-HCl buffer, shook it homogeneously, and then centrifuged.

Enzymatic Reactions of Organophosphates and Sulfonylurea Herbicides

As Table I shows, standard solutions of organophosphates (100 mg/L), Tris-HCl Buffer and enzyme solution were mixed and shaken homogeneously, then the reactions were carried out for 10 minutes under 37°C in pH 8.0 Tris-HCl buffer. Injected the reaction solutions into HPLC-UV and/or HPLC-MS system immediately and after 3, 6, 24, and 48 hours. The enzymatic reactions of sulfonylurea herbicides were the same to the reactions of organophosphates.

Enzymatic Reaction of Pyrethroids

Similar to organophosphates, 10 mg/L mixed standard solution of pyrethroids, Tris-HCl buffer and enzyme solution were mixed and

TABLE I Operation of the Degradation Reactions of Organophosphates

Code	Enzyme (μL)	Organophosphorous standard solution (μL)	Tris-HCl buffer (μL)
1	100	50	350
2	100	5	395
3	50	50	400
4	50	5	445
5	25	50	425
6	25	5	470
7	0	50	450
8	0	5	495

shaken homogeneously, then the reactions undertook for 10 minutes under 37°C. After 3, 6, 24, and 48 h, pipetted 100 μL reaction solution and added 15 mg anhydrous $MgSO_4$ and 200 μL hexane, vortexed 1 min and centrifuged for 3 min under 4000 r/min, then detected the upper solution by GC-ECD.

Reversed-phase HPLC/UV and HPLC/MS Conditions

Confirmatory run analysis was performed on an Agilent HPLC1100 series equipped with an auto-sampler, an UV detector and a mass selective ion detector. A 250 × 4.6 mm column packed with 5 μm XDB-C18 particles (Agilent, USA) was used. HPLC/UV conditions were as follows: mobile phase, methanol/water (v%), 0–3 min, 15/85, 3–12 min, methanol (v%) 15%–85%, keep to 27 min; injection volume, 10 μL; flow rate, 1.0 mL/min. MS conditions were as follows: ionization mode, electrospray; polarity, positive; nitrogen drying gas flow rate, 6 mL/min; nebulizer pressure, 40 psi; dry temperature, 350°C. scanned-mass range, m/z 100–500.

GC-ECD Conditions

Agilent 6890 gas chromatograph equipped with a ^{63}Ni ECD electron-capture detector and a fused-silica capillary column HP-5 (30 m × 0.32 mm) was used. The operating conditions were as follows: initial temperature, 120°C (1 min), increased at 30°C /min to 280°C, kept for 14 min; injector temperature, 290°C; detector temperature, 300°C; N_2 carrier gas; column linear velocity (μ = 1.0 mL/min); operated in the split mode; injection volume, 1 μL.

REFERENCES

[1] W. E. Lambert, M. Lasarev, and J. Muniz, *Environ. Health Perspectives*, **113,** 504 (2005).
[2] J. S. Fletcher, T. G. Pfleeger, and H. C. Ratsch, *Environ. Sci. Technol.*, **27,** 2250 (1993).
[3] M. M. D. Fonseka, K. Medagoda, and Y. Tillakaratna, *Human exper. toxicol.*, **22,** 107 (2003).
[4] S. Fragoeiro and N. Magan, *Environ. Microbiol.*, **7,** 348 (2005).
[5] P. P. Kanekar, B. J. Bhadbhade, and N. M. Deshpande, *Proceedings of the Indian National Science Academy, Part B: Biol.i Sci.*, **70,** 57 (2004).
[6] B. T. Allinson and K. LeJeune, *Abstracts of Papers, 224th ACS National Meeting, Boston, MA, United States*, **8,** 18 (2002).
[7] S. Mark, M. Ashok, and C. Wilfred, *Biotechnol Bioengine*, **76,** 318 (2001).
[8] Y. L. Yu, D. F. Fan, and H. X. Chen, *Chinese Advan. in Environ. Sci.*, **4,** 28 (1996).
[9] H. Hirofumi, N. Sawako, and N. Tomoaki, *Chemosphere*, **55,** 641 (2004).
[10] Y. H. Liu, Y. Liu, Z. S. Chen, J. Lian, X. Huang, and Y. C. Chung, *Enzyme and Microbial Technol*, **34,** 297 (2004).
[11] N. F. Wu, M. J. Deng, G. Y. Liang, X. Y. Chu, B. Yao, and Y. L. Fan, *Chinese Science Bulletin*, **49,** 1245 (2004).
[12] N. F. Wu, M. J. Deng, X. Y. Shi, G. Y. Liang, B. Yao, and Y. L. Fan, *Chinese Science Bulletin*, **49,** 268 (2004).
[13] M. C. Serdar, *US Patent,* **5,** 589 (1996).
[14] W. W. Mulbry and J. Karns, *Appl. Environ. Microbiol.*, **55,** 289 (1989).
[15] M. C. Serdar and T. D. Gibson, *Biores, Technol.*, **3,** 567 (1985).