Enantioselective Induction of a Glutathione-S-Transferase, a Glutathione Transporter and an ABC Transporter in Maize by Metolachlor and Its (S)-Isomer

Sen Pang*, Zhaojin Ran*, Zhiqian Liu*, Xiaoyu Song, Liusheng Duan, Xuefeng Li*, Chengju Wang*

College of Sciences, China Agricultural University & Engineering Research Center of Plant Growth Regulators, Ministry of Education, China Agricultural University, Beijing, People’s Republic of China

Abstract

The metabolism of chiral herbicides in plants remains poorly understood. Glutathione conjugation reactions are one of the principal mechanisms that plants utilize to detoxify xenobiotics. The induction by rac- and S-metolachlor of the expression of three genes, ZmGST27, ZmGT1 and ZmMRP1, encoding respectively a glutathione-S-transferase, a glutathione transporter and an ATP-binding cassette (ABC) transporter was studied in maize. The results demonstrate that the inducing effect of rac- and S-metolachlor on the expression of ZmGST27 and ZmGT1 is comparable. However, the inducing effect of rac-metolachlor on ZmMRP1 expression is more pronounced than that of S-metolachlor. Furthermore, vanadate, an ABC transporter inhibitor, could greatly reduce the difference in herbicidal activity between rac- and S-metolachlor. These results suggest that the ABC transporters may preferentially transport conjugates of rac-metolachlor, leading to a faster metabolism of the latter. Through comparing the expression of ZmGST27, ZmMRP1 and ZmGT1 after treatment by rac- and S-metolachlor, we provide novel insights into the metabolic processes of chiral herbicides in plants.

Introduction

Chirality is a common phenomenon in life sciences. More than 30% of currently used pesticides are chiral compounds, including synthetic pyrethroids, organophosphate insecticides, imidazolinone and chloracetanilide herbicides [1,2]. The percentage of chiral pesticides is increasing with the introduction of more complex structures [3]. Enantiomers are defined as molecules that contain chiral structures and mirror images of each other [4]. Enantiomers of chiral pesticides have identical physicochemical properties, but exhibit quite different biochemical activities because biochemical processes usually show high stereo- or enantioselectivity [5]. Over the past two decades, studies on enantiomers differing in biological properties have been widely reported [6], including their biodegradation [7,8], toxicities to non-target organisms [9–14], endocrine-disrupting activities [15–17], etc. However, the enantioselective physiological effects and toxicities of chiral herbicides in plants have not received as much attention as insecticides in animals [1]. Although the enantioselective phytotoxicity of several chiral herbicides in plants has been evaluated [2,4,6,18,19], the metabolic processes of chiral herbicides in plants remain poorly understood.

Metabolism of herbicides in plants can generally be divided into three phases. In phase I, the herbicide may be oxidized, reduced or hydrolyzed to introduce or reveal a functional group. In phase II, the herbicide is conjugated to glutathione, glucose or malonate by the respective transferase to form a water-soluble conjugate. In phase III, herbicide conjugates are transported from the cytosol to the vacuole for further degradation. Glutathione conjugation plays a major role in the resistance of plants to herbicides. There have been numerous reports concerning the role of glutathione S-transferases (GSTs) in conjugating xenobiotics and the role of tonoplast ATP-binding cassette (ABC) transporters in transporting glutathione conjugates (GS conjugates) into the vacuole [20–22]. In addition to ABC transporters, recent studies showed that glutathione transporters, located in the plasma membrane, are able to mediate the transport of both reduced glutathione (GSH) and GS conjugates [23,24]. Our earlier work found that the expression of a glutathione transporter gene isolated from maize, named ZmGT1, was inducible by herbicides atrazine and metolachlor, and the inducing effect of metolachlor in different maize cultivars was correlated to their tolerance to this herbicide, suggesting an involvement of ZmGT1 in the detoxification of xenobiotics by plants [25,26]. Co-induction of ZmGST27, ZmMRP1 and ZmGT1 in maize by xenobiotics further suggests that, in addition to GSH, GSTs and ABC transporters, glutathione transporters located in the plasma membrane are also an important component in the glutathione conjugation-related plant detoxification system [27].
Metolachlor is a widely used herbicide that inhibits the synthesis of fatty acids in broadleaf weeds [20]. It was first introduced into the market as a racemic product, which contains both R- and S-enantiomers present in an equal ratio [19]. Racemic metolachlor is currently being replaced by S-metolachlor, which contains approximately 90% S-isomers and has the same herbicidal effect as the former when used at 65% of its dosage [29]. The sorption and desorption of metolachlor in the soil have been studied, along with its dissipation properties and effects on non-target species [30–33]. However, only limited information is available on the enantioselective behavior of rac- and S-metolachlor in plants [2,19]. Our previous work tested the effect of metolachlor on the expression of a glutathione-S-transferase (ZmGST27), an ABC transporter (ZmMRP1) and a glutathione transporter (ZmGT1) in maize leaves, but the effect of S-metolachlor on the expression of these genes was not investigated. Therefore, in the present study and 1C), metolachlor allowed determination of their respective EC50 values (from 4 to 96 h), the induction of ZmGST27, ZmMRP1 and ZmGT1 in maize leaves after treatment by rac and S-metolachlor. The aim was to provide insights into the metabolism of chiral herbicides in plants.

Results

Induction of ZmGST27, ZmMRP1 and ZmGT1 Expression by rac- and S-metolachlor

The semi-quantitative RT-PCR results showed that the expression of ZmGST27, ZmMRP1 and ZmGT1 was promoted by both rac- and S-metolachlor after 48 h treatment. While no significant difference was found between rac- and S-metolachlor in increasing the transcript level of ZmGST27 and ZmGT1 (Fig. 1A and 1C), rac-metolachlor was found to be a stronger inducer for ZmMRP1 expression as compared to S-metolachlor (Fig. 1B).

A time course study was designed to further verify the differential up-regulation of ZmMRP1 expression by rac- and S-metolachlor in maize leaves. The expression level of ZmMRP1 analyzed at 4, 8, 24, 48, 72 and 96 h after treatment by rac- and S-metolachlor confirmed the initial finding. As shown in Fig. 2, while a consistent but moderate increase in ZmMRP1 transcript level was afforded by S-metolachlor during the entire treatment period (from 4 to 96 h), the induction of ZmMRP1 expression by rac-metolachlor was moderate during the first hours but became dramatic from 24 h of treatment onwards.

Effects of rac- and S-metolachlor on the Growth of Maize Shoot

Dose-response experiments in the presence of rac- and S-metolachlor allowed determination of their respective EC50 values on maize growth. The EC50 value of rac-metolachlor is about 2.1-fold that of S-metolachlor, indicating S-metolachlor is more efficient in inhibiting the growth of maize shoot than rac-metolachlor (Table 1). When used alone, vanadate, an inhibitor of ABC transporters, had no significant effect on the growth of maize shoots (results not shown). However, this same compound significantly enhanced the inhibitory effect of rac-metolachlor on maize growth, as judged by the decrease of its EC50 from 375 to 104 μM. By contrast, only a small reduction in the EC50 of S-metolachlor was found when vanadate was applied (Table 1). As a result, the ratio of EC50 values of rac-metolachlor and S-metolachlor decreased from 2.1 to 1.3 in the presence of the ABC transporter inhibitor.

Discussion

Studies on chiral pesticides started to appear in the early 1990s [4]. However, there is still a severe lack of knowledge about the metabolism of chiral herbicides in plants [28]. Metolachlor was introduced into the market in 1976 as a racemic product. It was later found that the two 1’S-isomers of metolachlor afforded most of its biological activity. Our earlier work has provided evidence for the involvement of a glutathione-S-transferase (ZmGST27), a glutathione transporter (ZmGT1) and an ABC transporter (ZmMRP1) in the detoxification of metolachlor in maize [25,26]. However, a comparative analysis of the expression patterns of GSTs, ABC transporters and glutathione transporters in the same plant tissue after exposure to metolachlor and its S-isomer was lacking.

In this work, we investigated the enantioselective induction of ZmGST27, ZmGT1 and ZmMRP1 in maize by rac- and S-metolachlor. The role of GSTs in the detoxification of certain herbicides has been known for many years. The activation of GST genes in response to biotic and abiotic stresses has also been reported previously [34,35]. The non-selective up-regulation of ZmGST27 expression by rac- and S-metolachlor is likely to be a general response of this gene to chemical stress.

Our earlier work has shown that ZmGT1 is able to mediate the uptake of both GSH and GS conjugates by yeast cells, suggesting a potential role of this gene in xenobiotic detoxification [25]. The fact that S-metolachlor, which is enantiomerically enriched with the biologically active 1’S-isomer, gives rise to similar level of up-regulation of ZmGT1 expression in comparison to rac-metolachlor implies that this glutathione transporter has no preference in transporting GS conjugates of R- and S-isomer of metolachlor.

It is known that ABC transporters of plants have a wider spectrum of substrates as compared to glutathione transporters [27] and they are able to transport GS conjugates as well as glucose conjugates of certain herbicides [36–38]. A smaller increase in ZmMRP1 transcripts by S-metolachlor as compared to rac-metolachlor suggests that ABC transporters may preferentially transport GS conjugates of R-isomers. This may cause accelerated degradation of R-isomers, leading to reduced herbi-
cidal activity of rac-metolachlor as compared to S-metolachlor. The dramatic decrease of EC_{50} value of rac-metolachlor and the quasi-equal growth inhibition effect between rac- and S-metolachlor in the presence of an ABC transporter inhibitor (vanadate) further support this hypothesis. However, further investigation is needed to obtain direct evidence for faster degradation of R-isomers. More work is also needed to find whether the stronger herbicidal activity of S-isomer is related solely to its slower metabolism in plants.

In summary, racemic metolachlor is currently being replaced by S-metolachlor in application around the world. Through comparing the expression of ZmGST27, ZmMRP1 and ZmGT1 after treatment by rac- and S-metolachlor, we have found that rac- and S-metolachlor display differential activation on one of the detoxifying genes, suggesting a possible link between ABC transporter activity and differential plant sensitivity to chiral herbicides.

Materials and Methods

Chemicals

Rac-metolachlor (95% purity) and S-metolachlor (96% purity) were kind gifts from Institute of Plant Protection, Chinese Academy of Agricultural Sciences. Sodium orthovanadate was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd.

Plant Material

Maize (Zea mays, cv Bainuo No.2) were grown on sand in the glasshouse as described previously [25]. Plants grown to the 3-leaf stage were transferred to a hydroponic culture (three plants per pot). Each pot contained 50 mL of nutrient solution. Plants were adapted for 7 d and the nutrient solution was changed every 2 d. After the 7 d adaptation period, rac- and S-metolachlor were added into the nutrient solution to a final concentration of 100 μM and the nutrient solution was changed daily. At the end of the treatment, leaves were harvested, immediately frozen in liquid nitrogen, and stored at −80°C.

RNA Isolation

Frozen leaf samples were ground into a fine powder in liquid nitrogen. Total RNA was isolated using TRIzol reagent (Invitrogen, USA), and then digested by DNase I (TakaRa, China). First strand cDNA was synthesized with AMV reverse transcriptase and Oligo(dT)_{15}, according to the manufacturer’s protocol of Reverse Transcription System (Promega, USA).

Semi-quantitative RT-PCR Analysis of Transcripts

Three primer pairs (ZmGST27-F: 5'- GAC CTG CTC CTC GCC TCC AA -3' and ZmGST27-R: 5'- GCT CCA GGG TGT CCA TAG CG -3'; ZmGT1-F: 5'- GTG CCG CAG TGG TGG TTC -3' and ZmGT1: 5'- GTG ACG ACG AAG GCC AGG -3'; ZmMRP1-F: 5'- CTA GAA TAT GAA ACA CCA GCC AAG -3' and ZmMRP1-R: 5'- CTG CAA TAA TGG TGG ATC ATG TTG -3') were designed from the ORF region of ZmGST27 (accession number AF244692), ZmGT1 (accession number FJ573212), and ZmMRP1 (accession number AY186244) respectively to amplify a single fragment for each gene. Another pair of primers (P3: 5'- GTT CCT TCT TGA TTC TAT GGG TGG -3' and P4: 5'- GTT AGC AGG CTG AGG CAC TTC TG-3) was used to amplify a fragment from maize 18S ribosomal RNA (accession number M62386), chosen as an internal reference. PCR amplification conditions were: 30 s at 95°C, 30 s at 62°C, 30 s at 72°C (30 cycles).

Growth Inhibition Tests

The effect of rac- and S-metolachlor on the growth of maize shoots was assessed using the dose-response test. Rac- and S-metolachlor were dissolved in methanol to make a stock solution (10,000 mg L⁻¹). The stock solution was then diluted to five serial concentrations (25, 50, 100, 200 and 400 μM) using distilled water. Triton X-100 was added at the rate of 0.5 mL L⁻¹ as a wetting agent. Maize seeds were sown in plastic pots (9 cm diameter, 10 seeds per pot) filled with sand (250 g). After applying herbicide solutions, the pots (three replicates for each concentration) were placed in a growth chamber (25±2°C, 80±5% relative humidity) and watered as needed. Shoot lengths were measured 4 d after sowing. A 2nd dose-response test of rac-metolachlor and S-metolachlor was performed in parallel in the presence of an ABC transporter inhibitor, vanadate (sodium orthovanadate), which was added to the herbicide dilutions to a final concentration of 100 μM. Controls were treated with vanadate only. The effective concentration of herbicide causing 50% reduction (EC_{50}) in shoot growth was calculated using the method of Hill ([26]).

Table 1. Effects of various chemicals on the growth of maize shoots (4 d after treatment).

| Herbicides              | Regression equation | EC_{50} (μM) | R²   |
|-------------------------|---------------------|--------------|------|
| Rac-metolachlor         | y=0.58ln(x)+3.25    | 374.51       | 0.91 |
| S-metolachlor           | y=0.60ln(x)+3.11    | 175.41       | 0.90 |
| Rac-metolachlor + vanadate | y=0.45ln(x)+2.34   | 184.40       | 0.97 |
| S-metolachlor + vanadate | y=0.68ln(x)+3.35   | 138.58       | 0.90 |

*Vanadate was applied at a constant concentration of 100 μM.

doi:10.1371/journal.pone.0048085.t001
length was determined from the dose-response curve by Probit Analysis using the SPSS (version 16.0).

Acknowledgments

The authors acknowledge the technical support of Dr Zhonghua Yang of the College of Sciences, China Agricultural University.

References

1. Sekhon BS (2009) Chiral pesticides. J Pestic Sci 34: 1–12.
2. Liu HJ, Huang RN, Xie F, Zhang SX, Shi J (2012) Enantioselective phytoxicity of metolachlor against maize and rice roots. J Hazard Mater 217–218: 330–337.
3. Liu WP, Gan J, Schlenk D, Jury WA (2005) Enantioselectivity in environmental safety of current chiral insecticides. Proc Natl Acad Sci USA 102: 701–706.
4. Qian HF, Hu HJ, Mao YY, Ma J, Zhang AP, et al. (2009) Enantioselective phytotoxicity of the herbicide imazaquin in rice. Chesmmer 76: 885–892.
5. Muller TA, Kohler HP (2004) Chirality of pollutants effects on metabolism and fate. Appl Microbiol Biotechnol 64: 300–316.
6. Qian HF, Lu T, Peng Xf, Han X, Fu ZW, et al. (2011) Enantioselective phytotoxicity of the herbicide imazaquin on the response of the antioxidant system and starch metabolism in Arabidopsis thaliana. PLoS ONE 6: 1–12.
7. Falconer RL, Bidleman TF, Szeto SY (1997) Chiral pesticides in soils of the Fraser Valley, British Columbia. J Agr Food Chem 45: 1946–1951.
8. Kurt-Karakus PB, Bidleman TF, Jones KC (2005) Chiral organochlorine pesticide signature in global background soils. Environ Sci Technol 39: 8671–8677.
9. Wang LM, Ye WH, Zhou SS, Lin KD, Zhao MR, et al. (2009) Acute and chronic toxicity of organophosphate monocrotophos to Daphnia magna. J Environ Sci Heal B 44: 38–43.
10. Xu C, Zhao MR, Liu WP, Wang JJ, Chen SW, et al. (2008) Enantioselective separation and zebrabith embryo toxicity of insecticide acetofenate. Chem Res Toxicol 47: 4236–4242.
11. Liu HJ, Xiong MY (2009) Comparative toxicity of racemic metolachlor and S-metolachlor to Chlorella pyrenoidosa. Aquat Toxicol 93: 100–106.
12. Vallotton N, Moser D, Eggen RIL, Junghans M, Chevre N (2008) Co-induction of a glutathione S-transferase after glyphosate treatment in maize. J Pestic Sci 34: 1–12.
13. Zhan XM, Liu HJ, Xiao YG, Liu WP (2006) A comparative study of rac and S-metolachlor on some activities and metabolism of silkworm, Bombyx mori L. Pestic Biochem Phys 85: 133–138.
14. Liu HJ, Ye WH, Zhan XM, Liu WP (2006) A comparative study of rac and S-metolachlor toxicity to Daphnia magna. Ecotox Environ Safe 63: 451–455.
15. Miyashita M, Shimada T, Nakagami S, Kuribara N, Miyagawa H, et al. (2004) Enantioselective recognition of mono-demethylated methoxychlor metabolites by the estrogen receptor. Chemosphere 54: 795–800.
16. Hoekstra PF, Burnison BK, Garrison AW, Neheli T, Muir DC (2006) Estrogenic activity of diocfol with the human estrogen receptor: isomer- and enantiomer specific implications. Chemosphere 64: 174–177.
17. Jin YX, Chen RJ, Sun LW, Wang WY, Zhou L, et al. (2009) Enantioselective induction of estrogen-responsive gene expression by perethrin enantiomers in embryo-larval zebrafish. Chemosphere 74: 1238–1244.
18. Liu W, Ye J, Jin MQ (2009) Enantioselective phytotoxic effects of chiral pesticides. J Agric Food Chem 57: 2087–2095.
19. Xie F, Liu HJ, Cai WD (2010) Enantioselective racemic metolachlor and S-metolachlor in maize seedlings. J Environ Sci Health B 45: 808–816.
20. Shechan TA, Meade G, Foley VM, Dowd CA (2001) Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J 360: 1–16.
21. Theodoulou FL, Clark JM, He X, Pallette KE, Hallahan DL (2003) Co-induction of glutathione-S-transferases and multidrug resistance associated protein by xenobiotics. Pest Manag Sci 59: 202–214.
22. Swarbreck D, Ripoll PJ, Brown DA, Edwards KJ, Theodoulou F (2003) Isolation and characterization of two multidrug resistance associated protein genes from maize. Gene 315: 153–164.
23. Zhang MY, Bourbouloux A, Cagano O, Shrikantani CV, Renusch D, et al. (2004) A novel family of transporters mediating the transport of glutathione derivatives in plants. Plant Physiol 134: 482–491.
24. Cagano O, Bourbouloux A, Chakraborty D, Zhang MY, Delrot S (2004) AO repetitive transfers glutathione derivatives and is induced by primisulfuron. Plant Physiol 135: 1378–1387.
25. Pang S, Li XF, Liu ZQ, Wang CJ (2010) ZmG7T1 transports glutathione conjugates and its expression is induced by herbicide atrazine. Prog Biochem Pharmacol 37: 1120–1127.
26. Pang S, Duan LS, Liu ZQ, Song XY, Li XF, et al. (2012) Metolachlor-induced ZmG7T1 expression in maize cultivars is correlated with their tolerance to the herbicide. J Food Agric Environ 10: 621–623.
27. Pang S, Duan LS, Liu ZQ, Song XY, Li XF, et al. (2012) Co-induction of a Glutathione-S-transferase, a Glutathione Transporter and an ABC Transporter in Maize by Xenobiotics. PLoS ONE 7(7): e40712. doi:10.1371/journal.0040712.
28. Liu WP, Tang ML (2011) Enantioselective Activity and Toxicity of Chiral Herbicides. Herbicides - Mechanisms and Mode of Action, Dr. Mohammed Nagib Hassenen (Ed.), ISBN: 978-953-307-744-4. Intech website. Available: http://www.intechopen.com/books/herbicides-mechanisms-and-mode-of-action/enantioselective-activity-and-toxicity-of-chiral-herbicides. Accessed 2010.
29. Bazer FF, Poiger T, Muller MD (2000) Changed enantiomer composition of metolachlor in surface water following the introduction of the enantiomerically enriched product to the market. Environ Sci Technol 34: 2690–2696.
30. Ding GW, Novak JM, Herbert S, Xing BS (2002) Long-term tillage effects on soil metolachlor sorption and desorption behavior. Chesmmer 48: 897–904.
31. Cao PY, Wang XY, Liu FM, Zhao E, Han JI (2008) Disipation and residue of S-metolachlor in maize and soil. Bull Environ Contam Toxicol 80: 391–394.
32. Cook ME, Moore PA (2008) The effects of the herbicide metolachlor on agnostic behavior in the Crayfish, Orconectes rusticus. Arch Environ Contort Toxicol 53: 94–102.
33. Pereira SP, Fernandes MAS, Martins JD, Santos MS, Moreno APJ, et al. (2009) Toxicity assessment of the herbicide metolachlor comparative effects on bacterial and mitochondrial model systems. Toxicol In Vitro 23: 1585–1590.
34. Edwards R, Dixon DP, Wallot V (2008) Plant glutathione S-transferases: enzymes with multiple functions in sickness and in health. Trends Plant Sci 15: 193–198.
35. Basantani M, Srivastava A, Sen S (2011) Elevated antioxidant response and induction of two-class glutathione S-transferase after glyphosate treatment in Ficus raduate (L.) Wilczek. Pestic Biochem Physiol 99: 111–117.
36. Yuan JS, Tranell PJ, Steward CN (2007) Non-target-site herbicide resistance: a family business. Trends Plant Sci 12: 6–13.
37. Klein M, Burla B, Martinho E (2006) The multidrug resistance-associated protein (MRP/ABCC) subfamily of ATP-binding cassette transporters in plants. FEBS Lett 580: 1112–1112.
38. Guillard C, Dufaure A, Tommasini R, Kreuze K, Anheim N, et al. (1994) A herbicide antidote (saliner) induces the activity of both the herbicide detoxifying enzymes and a vascular transporter for the detoxified herbicide. FEBS Lett 332: 219–221.