Detoxification of Herbicides in *Phragmites australis*[§]

Peter Schröder[a,*], Heiko Maier[a,b], and Reinhard Debus[b]

[a] Institute of Soil Ecology, GSF National Research Center for Environment and Health, D-85758 Oberschleißheim, Germany. E-mail: peter.schroeder@gsf.de
[b] Technical University Wiesbaden, Department of Chemistry, D-65428 Rüsselsheim, Germany

* Author for correspondence and reprint requests

Unintentional loss of herbicides into drainage ditches, shores or other waterbodies may cause large problems in farmland. Therefore strategies for the phytoremediation of agrochemicals and especially herbicides have become a topic of great interest in many agricultural areas. However, in order to establish effective biological pollution control, information on the detoxification capacity of riparian plants and aquatic macrophytes (*e.g., Phragmites australis*) is important to build up effective buffer stripes. We determined the detoxification capacity of *Phragmites australis* roots and leaves for the conjugation of agrochemicals to glutathione by assaying the model substrate CDNB as well as the herbicides fenoxaprop-P, propachlor, pethoxamid and terbuthylazine. Specific GST activities were always higher in the rhizomes ($6.78 \pm 0.88\ \mu$kat/mg protein for CDNB) than in leaves ($1.08 \pm 0.21\ \mu$kat/mg protein). The detoxification capacity is distributed across an array of GST isoforms. In summary, *Phragmites australis* seems to be efficient in herbicide detoxification and a good candidate for phytoremediation of effluents from agricultural sites.

Key words: Glutathione Conjugation, Phytoremediation, Enzyme Induction

Introduction

Both roots and leaves of numerous plant species have been described to possess elaborate detoxification mechanisms for organic xenobiotics, predominantly herbicides (Marrs, 1996; Schröder, 1997). It has been demonstrated that herbicide tolerance in crops as well as resistance in weeds is partially based on the presence of these enzyme systems. It has generally been accepted that the responsible enzymes, although not physiologically connected, form a putative metabolic cascade for the detoxification, break down and final storage of organic xenobiotics. Sandermann and coworkers (1997) have compared this network of reactions with a "green liver", in analogy of the mammalian system.

This cascade has first been described by Shimabukuro (1976) who subdivided xenobiotic plant metabolism into three distinct phases, *i.e.* (I) activation of the xenobiotic, (II) detoxification and (III) excretion, in analogy to human hepatic metabolism. The cascade comprises of activation reactions catalyzed by P450 monooxygenases and peroxidases (phase I), true detoxification reactions in phase II performed by glutathione- and glucosyl-transferases, rendering the compound less toxic due to conjugation, and a set of further reactions that include cleavage, secondary conjugation and the like in phase III. In the past, xenobiotic conjugation in plants has been investigated in depth for pesticides, and several isoforms of glucosyl-transferases, malonyl-transferases and glutathione *S*-transferases have been identified in crops (Lamoureux and Rusness, 1989). Sugars, amino acids or glutathione may be transferred to the activated xenobiotic depending on the structure of the molecule and its active sites. OH-, NH_2-, SH- and COOH-functions on a molecule usually trigger glycosyl-transfer mediated by glycosyl-transferases (GT, E.C. 2.4.1.x; Frear, 1976), whereas the presence of conjugated double bonds, halogen- or nitro functions determines glutathione conjugation catalyzed by glutathione *S*-transferases (GST, E.C. 2.5.1.18; Coleman *et al.*, 1997).

Recently Schröder and Collins (2002) have pointed out that it is crucial to know in the context of phytoremediation, which type of primary conjugation occurred, because this will determine the final fate of the compound (Frear, 1976).

Glutathione *S*-transferases (GST, E.C. 2.5.1.18) are dimeric enzyme proteins of phase II that conjugate electrophilic, hydrophobic substrates to the tripeptide glutathione (GSH). GSTs play a major role in the intracellular detoxification of a wide array of xenobiotics (Lamoureux and Rusness, 1989). They were the target enzymes of our investigation.

Materials and Methods

Plant material

For the experiments, adult *Phragmites australis* plants were grown in a pond in Mörlbach near Munich, Germany. Samples of rhizomes and leaves were harvested immediately before the experiments and frozen in liquid nitrogen.

Chemicals

Bovine serum albumin (BSA), CDNB (1-chloro-2,4-dinitrobenzene), glutathione *S*-transferase (rat liver), reduced glutathione (GSH), Nonidet P 40, PVP K 30 (polyvinylpyrrolidone) were obtained from Sigma Chemie (Steinheim, Germany). Fenoxaprop-P (FNP-P) and pethoxamid (PA) were from Ehrenstorfer (Augsburg, Germany). Propachlor (PCl), terbuthylazine (TBA) and 1-naphthylacetic acid (NAA) were from RDH (Taufkirchen, Germany). All other chemicals used were research grade commercial material.

Enzyme extracts

The frozen leaves and roots were homogenized as previously described (Schröder *et al.*, 1990) under liquid nitrogen with a mortar and pestle to a fine powder and extracted at 4 °C in ten volumes (w/v) 0.1 M Tris-HCl buffer, pH 7.8, containing 1% of soluble PVP K 30, 1% Nonidet P 40 and 5 mM ethylene-diamine-tetraacetic acid (EDTA). The crude extract was centrifuged at $20,000 \times g$ and 4 °C for 20 min. Proteins in the supernatant were precipitated by stepwise addition of solid ammonium sulfate to 40% and in a second step to 80% saturation. During the procedure, the pH value was maintained at 7.8 by drop-wise addition of 10 M sodium hydroxide (NaOH). After each step the extracts were centrifuged at $20,000 \times g$ and the pellets were resuspended in 1 ml of 20 mM Tris-HCl buffer, pH 7.8. The extracts were desalted and further purified by passing through gel filtration columns (PD 10, Pharmacia, Freiburg Germany).

Spectrophotometric enzyme assay and protein determination

Standard spectrophotometric tests followed the methods of Habig *et al.* (1974). For the determination of the conjugation of model compounds, aliquots of the enzyme extract were incubated with 0.1 M Tris-HCl buffer, pH 7.8, 1 mM GSH with 1 mM CDNB ($\varepsilon_{340\,nm} = 9.6$ mM^{-1} cm^{-1}) or 1 mM 1,2-dichloro-4-nitrobenzene (DCNB, $\varepsilon_{345\,nm} = 8.5$ mM^{-1} cm^{-1}), furthermore with *p*-nitrobenzyl chloride (NBC, $\varepsilon_{310\,nm} = 1.8$ mM^{-1} cm^{-1}), and *p*-nitrobenzoyl chloride (NBOC, $\varepsilon_{310\,nm} = 1.9$ mM^{-1} cm^{-1}) resulting in a total assay volume of 0.6 ml. Controls lacking enzyme extracts or GSH were measured. Enzyme activity is expressed in units of kat (katal). 1 kat represents the enzymatic formation of 1 mol of end product per s in the enzyme extracts. Protein contents were determined by the standard method of Bradford (1976) using bovine serum albumin (BSA) as a reference protein.

Kinetics/inhibition

Steady state kinetic measurements were performed at 25 °C in 0.1 M Tris-HCl buffer. Michaelis-Menten constants for CDNB, DCNB and GSH were determined from Lineweaver-Burk plots. The measurements were performed according to the standard assay procedures cited above. The concentration of the substrates was varied in a range from 0.0625 to 1 mM. The enzymatic rate of conjugation was corrected for non-enzymatic rates.

Substrate inhibition was assayed using the herbicides fenoxaprop-P (FNP-P), propachlor (PCl), pethoxamid (PA) and terbuthylazine (TBA). The concentration of the inhibitors was varied in a range from 0.0625 to 1 mM. Also in this case, GST activity for CDNB was determined at 25 °C following the standard spectrophotometric test procedures (Habig *et al.*, 1974).

Induction of GST

Detoxification enzymes in *Phragmites australis* rhizomes were induced following the protocol of Robineau and coworkers (1998). For aging experiments, 1 g of rhizome was sliced (1.5 mm thick), washed, and incubated in 5 ml aerated distilled water. Several inducers were added in 1 ml of water and the induction experiments were started in Petri dishes for 1, 6, 24 h at 4 °C in the dark.

The concentration of the stock solutions was 30 mM propachlor (in EtOH) and 30 mM fenoxaprop-P (in EtOH), and 10 mM Bion (benzothiadiazole, in destilled H_2O) and 10 mM naphthylacetic acid (in EtOH). The extraction of the enzymes followed the protocol described above. Photometric determination of enzyme activities after the induction was accomplished with the model GST-substrates CDNB, NBC, propachlor and NBOC.

HPLC

High-performance liquid chromatography (HPLC) of herbicide metabolites was performed with a Varian Model 230 HPLC System equipped with a Gynkotek Spectrometer SP-4 UV monitor at 254 nm. For HPLC analyses, a RP-C_{18} Hypersil-ODS-column, 5.0 μm, 250 × 4.0 mm, radial cartridge (Bischoff) was used with acetonitrile/water with 0.1% trifluoroacetic acid solvent system. Peaks were eluted at a flow rate of l ml/min in a gradient from 60% acetonitrile for 5 min followed by a 15 min increase to 100% acetonitrile.

Affinity chromatography

In order to separate GST isoenzymes from the crude extracts, samples were loaded onto GSH agarose and *S*-hexyl agarose columns (Pharmacia, Germany) pre-equilibrated with 20 ml 25 mM Tris-HCl, pH 7.8. After use the column was washed with five volumes of the buffer. Bound GST was eluted by a stepwise gradient of 0 to 16 mM GSH dissolved in 25 mM Tris-HCl buffer with the addition of 0.1 to 0.5 M NaCl. To concentrate the protein in fractions (1 ml) containing GST activity, the buffer-salt mix was exchanged against water by gel filtration on Sephadex G25 columns and protein was lyophilized over night. GST activity was tested using the CDNB-standard test.

Gel electrophoresis

Molecular mass and purity of the GST isoenzymes were estimated by SDS-PAGE using 8 to 20% gradient gels. A rat GST standard (Sigma, Dreieich, Germany) was used as a standard and a low molecular weight marker for determination of the GST molecular weights (14–116 kDa). Lyophilized proteins were resuspended in water. Prior to electrophoresis samples were diluted 1:1 with loading buffer and proteins were denatured by 95 °C for 5 min. Gels were silver stained following the method of Blum and coworkers (1987).

Results

Enzyme activity

Glutathione *S*-transferase activity for the model substrate CDNB was found in leaves and rhizomes of *Phragmites*, in accordance to Pietrini and coworkers (2003) (Table I). In addition, activity for the conjugation of the closely related DCNB has been detected. Rhizomes exhibit 10-fold higher GST activities than leaves, due to the lower protein contents (0.85 mg/g FW as compared to 9.08 mg/g FW) of the storage tissue. However, the high GST activity in rhizomes points to the importance of the enzyme in root metabolism and defence against foreign compounds.

As it is known from previous studies that GST isoforms in different plant tissues are not identical, which results in differences in the substrate spectrum, Michaelis-Menten kinetics were recorded. Whereas the K_M-values for CDNB are equal in both rhizomes and leaves, significant differences have been found for the substrate DCNB. Furthermore, the affinity of the rhizome enzyme versus the cosubstrate, glutathione, is higher than in leaves. Interestingly, the K_M- and v_{max}-values for the herbicide propachlor show a similar tendency. They were lower in the leaves but extremely higher in the rhizomes than for CDNB. The K_M-value for terbuthylazine was found to be 1.34 mM in the rhizomes (Table II). Mc Gonigle and coworkers (2000) reported similar catalytic properties in maize and soybean. Their findings confirm large differences in specific activities and also conjugative activities higher for herbicides than for the standard substrate CDNB.

These results stimulated further studies on the enzyme kinetics, this time using the herbicides as inhibitors of CDNB conjugation. Inhibition kinetics allow for the distinction between enzyme isoforms or at least clusters of enzymes (Segel, 1975).

Table I. The comparison between the GST activity from leaf- and root extracts of *Phragmites australis* (the concentration of the substrates was 1 mM CDNB and 1 mM GSH, the GST activity was measured with a standard test).

	Enzyme activity [μkat/g FW]	Protein concentration [mg/g FW]	Specific activity [μkat/mg protein]
Leaves	11.62	9.08	1.08 ± 0.21
Rhizomes	6.52	0.85	6.78 ± 0.88

Table II. K_M- und v_{max}-values for the extracted enzymes of *Phragmites* rhizomes and leaves. Conjugation was performed with glutathione, the concentration of the co-substrate was constant. K_M for GSH was determined under conditions of constant CDNB concentrations. R^2 values were generally above 0.9. All measurements were done in triplicate; n.d., not determined.

	Leaves		Rhizomes	
	K_M [mM]	v_{max} [μkat/mg protein]	K_M [mM]	v_{max} [μkat/mg protein]
CDNB	0.83	1.58	0.82	8.98
GSH/CDNB	0.19	0.86	0.16	5.15
DCNB	0.11	0.046	0.26	0.11
Propachlor	0.088	0.069	2.68	17.64
Terbuthylazine	n.d.	n.d.	1.34	0.70

Fig. 1 depicts results of inhibition studies of CDNB conjugation in the presence of herbicides. It had previously been concluded from inhibition kinetics of this kind that the conjugation might be competitively inhibited by herbicides (Schröder, 1997). Using fenoxaprop-P as an inhibitor of *Phragmites* GST at three different concentrations resulted in a typical Dixon plot with a clear interception above the *x*-axis. This is indicative of a classical competitive inhibition in leaves with a K_I of 0.55 mM and in rhizomes with a K_I of 0.09 mM (Table III). Contrary to this, increasing inhibitor concentrations of the herbicides pethoxamid, propachlor and terbuthylazine do not result in straight first order kinetics, but in asymptotic curves. From these curves, at least two derivative lines can be drawn, resulting in two distinct K_I-values for the reaction under consideration. The K_I-values in leaves are 1.56 mM and 2.65 mM with

Table III. K_I-values show the inhibition for the extracted enzymes of *Phragmites* rhizomes and leaves. The concentration of the inhibitors was varied in a range from 0.0625 to 1 mM. GST activity was determined following the standard test. R^2 values were generally above 0.9. All measurements were done in triplicate; n.d., not determined.

		Leaves K_I [mM]	Rhizomes K_I [mM]
Fenoxaprop-P	K_I	0.55 ± 0.12	0.09 ± 0.03
Pethoxamid	K_{I1}	1.56 ± 0.34	2.15 ± 0.37
	K_{I2}	2.65 ± 0.77	3.07 ± 0.98
Propachlor	K_{I1}	0.56 ± 0.03	n.d.
	K_{I2}	1.96 ± 0.14	n.d.
Terbuthylazine	K_I	n.d.	1.12 ± 0.32

pethoxamid, and 0.56 mM and 1.96 mM with propachlor. The K_I-values in rhizomes are 2.15 mM and 3.07 mM with pethoxamid and 1.12 mM with terbuthylazine. This is indicative of the presence of several sets of isoenzymes in leaves and rhizomes with different inhibitor constants in the extracts used for the experiments. Similar results have been presented for the GSTs in tree species (Schröder and Götzberger, 1997).

Induction studies

In order to obtain information on the possible enhancement of *Phragmites* GST by chemicals, rhizomes of the plant were incubated with benzo-thiadiazole (Bion), naphthylacetic acid (NAA), and the herbicides fenoxaprop-P and propachlor. It was speculated that the reaction of GST activity on these chemicals would allow for recommendations how to increase the detoxification capacity of the reed plant. Further it was assumed that the plant activators would increase GST more rapidly and stronger than the herbicides under consideration. GST activities in the rhizomes were determined with CDNB, propachlor, NBC and NBOC after 1, 6 and 24 h of incubation.

Benzothiadiazole, a known inducer of plant resistance and marketed as Bion to improve plant performance (Fig. 2), exerted significant effects on three of the chosen substrates, whereas NBOC conjugation was not affected in any experiments. After 1 h of incubation, the GST activity in reed rhizomes was even found to be slightly lower than in untreated controls, and after 6 h, only NBC conjugation was increased above controls. After 24 h, however, CDNB conjugation was increased 6-fold and the formation of propachlor conjugates was enhanced by a factor of 25.

Naphthylacetic acid, a known herbicide safener, led to contrasting effects. CDNB and NBOC conjugations were enhanced only during the first 6 h of the incubation period. However, the induction effect was lost after 24 h. Both, propachlor and NBC conjugation were inhibited throughout the experiment.

In contrast to these experiments with known inducers, the following two induction studies used herbicides as active agents (Fig. 3). Fenoxaprop-P caused huge inductions of 23-fold and 15-fold for the GST activities conjugating CDNB and propachlor, but no effect was recorded for the other nitrobenzenes. It is remarkable that the reaction is
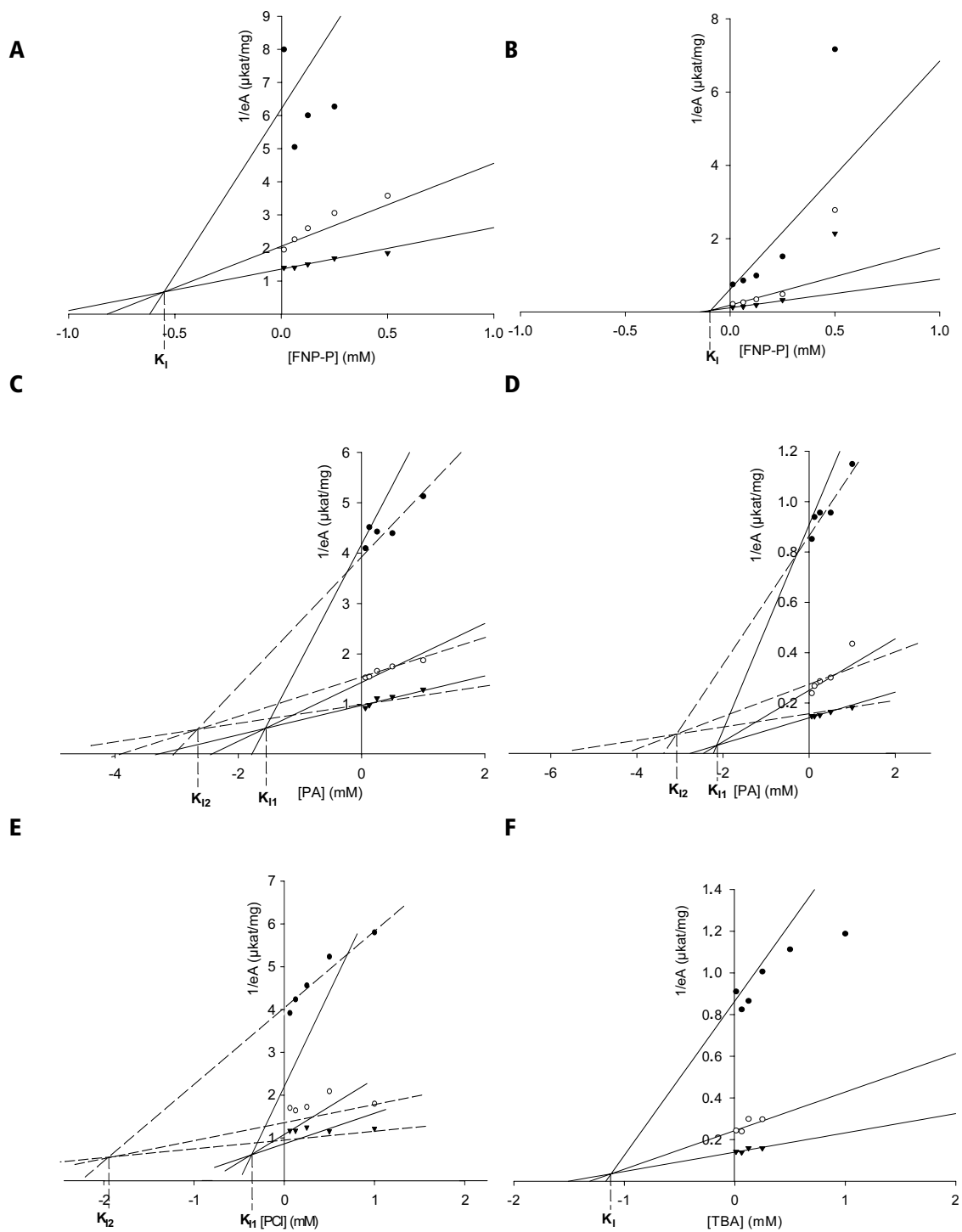
Fig. 1. Inhibition of the GST activities from *Phragmites australis.* (A) GST inhibition in leaves by fenoxaprop-P; (B) inhibition in tubers by fenoxaprop-P; (C) GST inhibition in leaves by pethoxamid; (D) inhibition of GST in tubers by pethoxamid; (E) inhibition of GST in reed leaves by propachlor; (F) inhibition of GST in tubers by terbuthylazine.
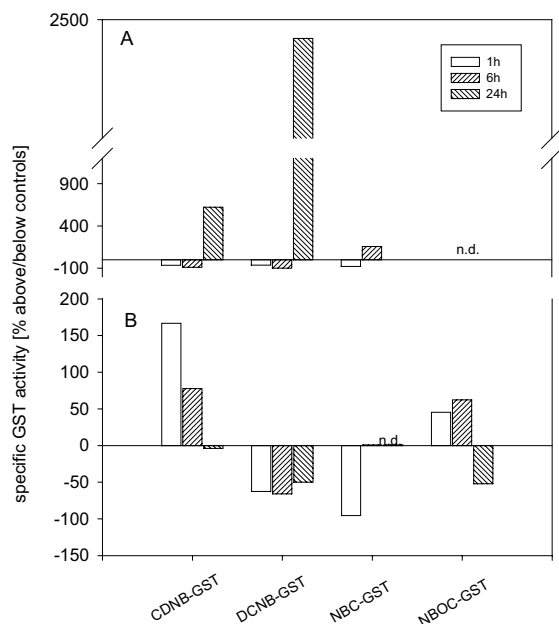
Fig. 2. GST activity in *Phragmites* rhizomes following incubation with known inducers relative to untreated controls. Rhizome fractions were incubated for 24 h with (A) Bion and (B) NAA in given concentrations. Samples were taken after 1 h, 6 h and 24 h. The rhizomes were frozen in liquid nitrogen and stored at −80 °C. Enzyme extraction was according to the Materials and Methods section and GST activity for several model compounds and the herbicide propachlor was determined. All experiments were performed in triplicate.
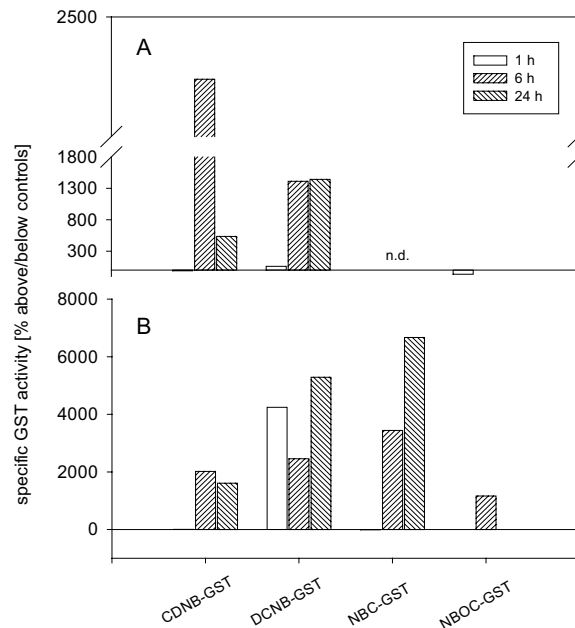
Fig. 3. GST activity in *Phragmites* rhizomes following incubation with known inducers relative to untreated controls. Rhizome fractions were incubated for 24 h with (A) fenoxaprop-P and (B) propachlor in given concentrations. Samples were taken after 1 h, 6 h and 24 h. The rhizomes were frozen in liquid nitrogen and stored at −80 °C. Enzyme extraction was according to the Materials and Methods section and GST activity for several model compounds and the herbicide propachlor was determined. All experiments were performed in triplicate.

seen only after 6 h of incubation, whereas NAA led to strong effects from the onset of the incubation.

Overall, the chloroacetanilide herbicide, propachlor, caused the strongest induction in all incubations. After one hour, propachlor conjugation was already enhanced 40-fold in the treated plants, and after 6 h, 10- to 20-fold enhanced conjugation was measured for all substrates under consideration. At the end of the experiments, after 24 h, up to 70-fold increased conjugation rates were found for propachlor and NBC, a substrate that is not very actively conjugated under control conditions. Again, NBOC seems to reflect the weakest effects of all xenobiotic substrates tested. These results are of special importance, as Dixon *et al.* (2003) were able to show, that plant Phi GSTs exhibit high activities for the conjugation of chloroacetanilides, whereas GSTs of the Tau family are active against aryloxyphenoxy-propionic acids.

Furthermore our data indicate that there are several isoforms of GST present in reed rhizome. Whereas some isoforms seem to have overlapping substrate specificities, the nitrobenzylchlorides seem to be substrates to distinct GST groups.

This assumption is also underlined by our attempts to further purify reed rhizome GSTs by affinity chromatography. On an *S*-bound glutathione agarose gel, GSTs elute in four distinct peaks at different GSH and NaCl concentrations with 90% recovery (Fig. 4). Similar results have been reported for wheat GST by Cummins and coworkers (1997) thus indicating differences in affinity to the substrate, *i.e.* differences in the sequence of the binding site, as well as differences in the charge of the enzyme, *i.e.* differences in the tertiary structure.

Gel electrophoretic separation of the proteins in these peaks confirmed the presence of several bands in the area of 25 to 30 kDa, the typical GST subunit weight (data not shown).
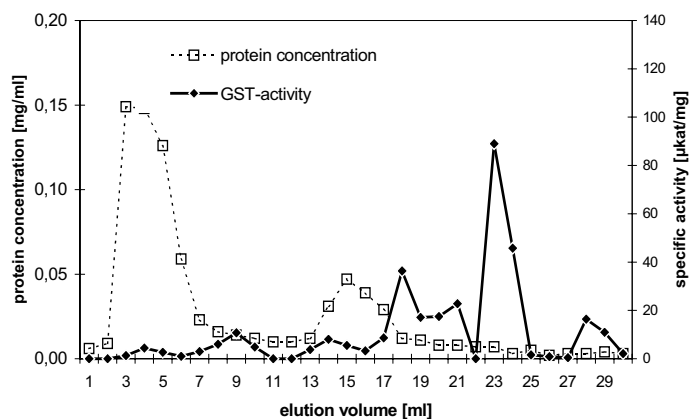
Fig. 4. Elution profile of *Phragmites*-GSTs from a GSH(S)-agarose-column. GST activity elutes in a continuous GSH-NaCl-gradient applied after fraction 15. Four distinct groups of GST isoforms with CDNB activity are eluted.

Conclusion

Agricultural practise frequently leads to pollution of adjacent water bodies. *Phragmites australis* can be used to diminish this load with agrochemicals as it is able to detoxify a whole array of xenobiotics, including herbicides. Besides its high basic activity for the conjugation of these compounds, the tested plants exhibited also extremely good inducibility of the detoxification enzymes. However, typical inducers of resistance were only active with specific GSTs and caused also inhibition rather than enhancement of activity. Our studies led to the assumption that there are different induction mechanisms for the GSTs under consideration and our data shed some light on the possible inhibition of the enzymes under the influence of xenobiotics. Such a negative effect of xenobiotics on the detox-

ification capacity would also negatively influence phytoremediation, and the presence or absence of certain ingredients in an effluent mixture would well be decisive for a successful treatment.

The herbicides fenoxaprop-P and propachlor were by far the most potent inducers and activated the whole array of tested GSTs. Our results demonstrate that several induction mechanisms are present in the reed plant. Oxidative stress caused by the action of the used herbicides might be one of the most potent triggers for GST induction.

Overall reed seems to be a good candidate for phytoremediation of organic xenobiotics from water bodies. Further studies must include the role of the leaves in phytoremediation and try to elucidate the induction mechanisms as well as the GST family structure in *Phragmites*.

Blum H., Beier H., and Gross H. (1987), Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis **8**, 93–99.

Bradford M. M. (1976), A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. **72**, 248–255.

Coleman J. O. D., Blake-Kalff M. M. A., and Davies T. G. E. (1997), Detoxification of xenobiotics by plants: Chemical modification and vacuolar compartmentation. Trends Plant Sci. **2**, 144–151.

Cummins I., Cole D. J., and Edwards R. (1997), Purification of multiple glutathione transferases involved in herbicide detoxification from wheat (*Triticum aestivum* L.). Pestic. Biochem. Physiol. **59**, 35–49.

Dixon D. P., Mc Ewen A. G., Lapthorn A. J., and Edwards R. (2003), Forced evolution of a herbicide detoxifying glutathione transferase. J. Biol. Chem. **278**, 23930–23935.

Frear D. S. (1976), Pesticide conjugation-glycosides. ACS Symposium Ser. **29**, 35–54.

Habig W. H., Pabst J., and Jakoby W. B. (1974), Glutathione *S*-transferases, the first step in mercapturic acid formation. J. Biol. Chem. **249**, 7130–7139.

Lamoureux G. L. and Rusness D. G. (1989), The role of glutathione and glutathione *S*-transferase in pesticide metabolism, selectivity and mode of action in plants and insects. In: Glutathione: Chemical, Biochemical and Medical aspects (Dolphin D., Poulson R., and Avramovic O., eds.). Series: Enzyme and Cofactors. John Wiley and Sons, New York, pp. 155–195.

Marrs K. A. (1996), The functions and regulation of glutathione *S*-transferases in plants. Annu. Rev. Plant Physiol. **47**, 127–158.

Mc Gonigle B., Keeler S. J., Lau S. M., Koeppe M. K., and O'Keefe D. P. (2000), A genomics approach to the comprehensive analysis of the glutathione *S*-transferase gene family in soybean and maize. Plant Physiol. **124**, 1105–1120.

Pietrini F., Iannelli M. A., Pasqualini S., and Massacci A. (2003), Interaction of cadmium with glutathione and photosynthesis in developing leaves and chloroplasts of *Phragmites australis* (Cav.) Trin. ex Steudel. Plant Physiol. **133**, 829–837.

Robineau T., Batard Y., Nedelkina S., Cabello-Hurtago F., Le Ret M., Sorokine O., Didierjean L., and Werck-Reichardt D. (1998), The chemically inducible plant cytochrome P450 CYP76B1 actively metabolizes phenylureas and other xenobiotics. Plant Physiol. **118**, 1049–1056.

Sandermann H., Haas M., Messner B., Pflugmacher S., Schröder P., and Wetzel A. (1997), The role of glucosyl and malonyl conjugation in herbicide selectivity. In: Regulation of Enzymatic Systems Detoxifying Xenobiotics in Plants (Hatzios K. K., ed.). Kluwer Academic Publisher, Dordrecht, pp. 211–231.

Schröder P. (1997), Fate of Glutathione *S*-conjugates in plants: Cleavage of the glutathione moiety. In: Regulation of Enzymatic Systems Detoxifying Xenobiotics in Plants (Hatzios K. K., ed.). Kluwer Academic Publishers, Dordrecht, pp. 233–244.

Schröder P. and Götzberger C. (1997), Partial purification and characterization of glutathione *S*-transferse isozymes from the leaves of *Juniperus communis*, *Larix decidua* and *Taxus baccata*. J. Appl. Bot. Food Qual. **71**, 31–37.

Schröder P. and Collins C. (2002), Conjugating enzymes involved in xenobiotic metabolism of organic xenobiotics in plants. Int. J. Phytorem. **4**, 247–265.

Schröder P., Lamoureux G. L., Rusness D. G., and Rennenberg H. (1990), Glutathione *S*-transferase activity in spruce needles. Pestic. Biochem. Physiol. **37**, 211–218.

Segel I. H. (1975), Enzyme Kinetics, Behaviour and Analysis of Rapid Equilibrium and Steady-state Enzyme Systems. Wiley-Interscience, Toronto.

Shimabukuro R. H. (1976), Glutathione conjugation of herbicides in plants and animals and its role in herbicidal selectivity. Asian-Pac. Weed Sci. Soc., 183–186.