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The Induction of Glutathione S-Transferase and C-S Lyase in the Needles of Spruce Trees

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Summary

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Xenobiotic substances appear to be detoxified and metabolized in needles and callus cultures of Norway spruce (*Picea abies* [L.] Karst.) via a pathway including glutathione S-transferase (GST), peptidases, cysteine S-lyase (CS-lyase) and S-adenosyl-methionine-methyltransferase (SAM-methyl-transferase) enzymes. In the present study, the induction of GST and CS-lyase by xenobiotics was investigated in spruce twigs. GST activity in the needle extracts seemed to be related to the presence of several isozymes, the induction of which was not interrelated. CS-lyase activity was increased by pretreatment with every substrate used.

Introduction

The emission of organic xenobiotics from anthropogenic sources and their deposition in the biosphere has been the subject of many studies (PATERSON & al. 1990, MAJEWSKI 1991). Data on the fate of these substances in plants, however, are scarce. Information is especially lacking for plants without direct nutritional use for animals or man. Recent publications (SCHRÖDER & al. 1990a,b, LAMOUREUX & al. 1991) showed that several xenobiotics were metabolized by the

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action of glutathione S-transferases (GST) in the needles of Norway spruce (*Picea abies* [L.] KARST.) and two North American spruce species (*P. glauca* [L.] Moench. and *P. pungens* [L.] Engelm.). The activity of GST in Norway spruce needles varies greatly among the substrates tested. Maximal activity was observed with 1-chloro-2,4-dinitrobenzene, but fluorodifen, a diphenyl-ether herbicide was also a very effective substrate (SCHRÖDER & al. 1990a). In spruce, the fluorodifen glutathione-conjugate was rapidly cleaved to the cysteine conjugate (SCHRÖDER & al. 1990b) which was metabolized further by several routes (LAMOUREUX & al. 1991). One of these routes appeared to involve cysteine S-lyase (CS-lyase) and S-adenosyl-methionine-methyltransferase (SAM-methyl-transferase) enzymes present in the needles. The purpose of the present study was (a) to quantify the effect of three xenobiotics on the levels of GST and CS-lyase activity and (b) to elucidate the possible involvement of different GST isozymes in the detoxification process.

Materials and Methods

The experiments were carried out using needles and cuttings of cloned Norway spruce trees (*Picea abies* L. Karst, cv. Vorallgäuer Fichte), grown at sites in Garmisch-Partenkirchen.

The metabolic pathway was investigated in branch cuttings harvested from cloned six year old spruce trees as previously described (SCHRÖDER & al. 1992). Distal twigs bearing the youngest needle year class (1991) were harvested from the trees as cuttings and immediately immersed into water to prevent embolies. At the beginning of the experiments, the cuttings (approx. 2 to 3 g fresh weight) were incubated with 2 ml 100 mM potassium phosphate buffer (pH 7.8), containing either 2 mM p-nitrobenzyl chloride PNBC, S-(4-nitrophenyl)-glutathione (NP-SG), or S-(2,4-dinitrophenyl)-cysteine (DNP-CYS). After 15 h at room temperature and a light intensity of 650 µmol m^{-2} s^{-1}, the incubations were stopped by withdrawing the buffer solution and freezing the cuttings in liquid nitrogen.

Enzyme extracts were prepared from spruce needles and assayed for GST activity in 100 mM potassium phosphate buffer at pH 6.4; 1 mM 1-chloro-2,4-nitrobenzene (CDNB, $e_{340\ nm}$ mM^{-1} cm^{-1} = 9.6; HABIG & al. 1974), dissolved in EtOH; and 1 mM reduced glutathione, (GSH) (SCHRÖDER & al. 1990a). The reaction was started by the addition of varying amounts of enzyme. Controls lacking GST or GSH were measured. Correspondingly, p-nitrobenzyl chloride (PNBC, $e_{310\ nm}$ mM^{-1} cm^{-1} = 1.9) and 1,2-dichloro-4-nitrobenzene (DCNB, $e_{345\ nm}$ mM^{-1} cm^{-1} = 8.5) were used as substrates as described by HABIG and coworkers 1974. These enzyme extracts were also assayed for CS-lyase activity in 100 mM potassium phosphate buffer at pH 7.5; 0.1 mM S-(2,4-dinitrophenyl)-cysteine (DNP-CYS, $e_{400\ nm}$ mM^{-1} cm^{-1} = 20) and an aliquot of the enzyme extract using the method of HANSEN & al. 1959. Protein in the extracts was determined according to BRADFORD 1976. Experimental data were subjected to statistical testing using PC based Statgraf™ software.

Results and Discussion

CS-lyase activity in protein extracts derived from *P. abies* needles was measured using DNP-CYS. The reaction was linear for at least 5 min and was dependent on the amount of protein present in the assay (Fig. 1). The specific activity was approximately 0.7 nmol mg $protein^{-1}$ min^{-1}. Very little CS-lyase activity was

observed in the boiled enzyme and no-enzyme controls and addition of dithiothreitol (DTT) had little effect on the enzyme assay during the first 5 min.
Fluorodifen was shown to be metabolized in spruce cell cultures and spruce needles to a glutathione conjugate which was subsequently converted to a cysteine conjugate and a variety of other metabolites (SCHRÖDER & al. 1990a,b, LAMOUREUX & al. 1991). It was speculated that among the products formed from the cysteine conjugate were 4-trifluoromethyl-2-nitrothiophenol and 4-trifluoromethyl-2-nitrothioanisole (LAMOUREUX & al. 1991). In this study, incubation of spruce needle extracts with 0.1 mM cysteine conjugate resulted in the formation of these products at initial rates of 7.7 nmoles g fresh weight^{-1} h^{-1} of 4-trifluoromethyl-2-nitrothiophenol and 0.74 nmoles g fresh weight $^{-1}$ h^{-1} of 4-trifluoromethyl-2-nitrothioanisole with the addition of 1 mM DTT and 0.1 mM SAM cofactors. These results indicated the presence of GST, CS-lyase and SAM-CH_3-transferase activity in spruce needles.

To test the effect of xenobiotics or xenobiotic metabolites on this pathway, spruce needles were incubated with PNBC, the glutathione conjugate NP-SG, and the cysteine conjugate DNP-CYS for 15 h prior to extraction of the enzymes. The amounts of xenobiotics, especially of NP-SG and DNP-CYS, which were actually taken up by the needles were not determined. However, as 1 to 1.5 ml of the incubation buffer containing the xenobiotics was taken up by each cutting during the experiments (Table 1A), it may be assumed that uptake of xenobiotics was 2 to 3 µmol in 15 h.

Table 1. Uptake and effect of xenobiotics after 15 h incubations. A: uptake of incubation buffer during 15 h, B: activity of GST and CS-lyase after incubation with the xenobiotic substances PNBC, NP-SG and DNP-CYS (1 µkat l^{-1} = 16.67 nmol ml^{-1} min^{-1}, BERGMEYER 1983). CON = control cuttings without xenobiotic treatment. Data are means ± standard deviations of 3 independent samples from 2 to 3 replicate experiments.

	Treatment			
	PNBC	NPSG	DNPCYS	CON
(A)	Uptake (ml 15 h^{-1})			
	1.51 ± 0.50	1.34 ± 0.64	1.62 ± 0.65	1.26 ± 0.14
(B)	Enzyme Activity (µkat l^{-1})			
GST:CDNB	1.84 ± 0.36	1.51 ± 0.54	1.69 ± 0.26	0.77 ± 0.11
GST:DCNB	0.14 ± 0.04	0.32 ± 0.16	0.22 ± 0.18	0.34 ± 0.15
GST:PNBC	0.30 ± 0.08	0.06 ± 0.04	0.10 ± 0.08	0.23 ± 0.09
CS-LYASE	0.12 ± 0.06	0.09 ± 0.04	0.08 ± 0.03	0.05 ± 0.03

The GST and CS-Lyase activities in the untreated cuttings were in the range of previously recorded values, but changed significantly in the treated samples (Table 1B, Fig. 2).

Treatment with PNBC caused a doubling of CDNB/GST activity and a 50% decrease in DCNB/GST activity. Incubation of cuttings with the NP-SG or the DNP-CYS resulted in a 100 % increase of CDNB/GST activity and a significant decrease in PNBC/GST activities (Fig. 2B,C). There appeared to be an inverse relationship between CDNB/GST activity and DCNB/GST activity; when CDNB/GST activity was stimulated DCNB/GST activity was decreased. PNBC conjugation was slightly enhanced by pretreatment with PNBC but was not significant; it was diminished significantly after preincubation with NP-SG or DNP-CYS. These results suggest that spruce contains several GST-isozymes, the activity of which can be selectively stimulated or inhibited as has been demonstrated in sorghum (DEAN & al. 1990). Although it is not known whether the observed changes in GST activity were due to changes in *de novo* synthesis in response to a xenobiotic stressor, recent results with spruce indicate that GST might be induced rapidly and very effectively (SCHRÖDER & al. 1992).

CS-lyase activity responded in a manner very similar to CDNB/GST activity. It was enhanced after pre-treatment with PNBC, NP-SG, and DNP-CYS (Fig. 2). It cannot be determined from this study whether the CS-lyase activities increase in response to the initial xenobiotic substrate used for incubation or a metabolite formed *in vivo* during the treatment, such as the cysteine conjugate, that is produced from that xenobiotic.

In conclusion, a metabolic pathway for xenobiotic nitrophenyl compounds in spruce needles appears to proceed via GST, peptidases, CS-lyase and SAM-transferase. Similar to other plants, spruce needles appear to possess multiple GST isozymes the activity of which is induced by different substrates. CS-lyase activity also appears to be inducible, and responds similar to CDNB/GST. The presence, activity and possible induction of peptidases and SAM-methyltransferases in spruce needles is still subject to speculation, but this pathway would be in accordance with previous results from metabolism studies in onion roots (LAMOUREUX & RUSNESS 1980). In that study, peptidase and CS-lyase activities were reported to be extremely high, and SAM-methyl-transferase activity was found to be the rate limiting step of the metabolic pathway of pentachloronitrobenzene. Further studies should focus on the CS-lyase and SAM-transferase activities in spruce needles in order to describe the dynamics of the proposed pathway.

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References

BRADFORD M. M. 1976. - Anal. Biochem. 72: 248-254.

BERGMEYER H. U. 1983. - Methods of Enzymatic analysis I, Vch Weinheim, pp. 12ff.

DEAN J. V., GRONWALD J. W. & RENNENBERG C. V. 1990. - Plant Physiol. 92: 467-473.

HABIG W. H., PABST M. J. & JAKOB W. B. 1974. - J. Biol. - Chem. 249: 7130-39.

HANSEN S. E., KJAER, A. & SCHWIMMER S. 1959. - C.R. Trav. Lab. Carlsberg, Ser. Chim. 31: 193-202

LAMOUREUX G. L. & RUSNESS D. G. 1980. - Pest. Biochem. Physiol. 14: 50-61.

— , — , *SCHRÖDER P. & RENNENBERG H. 1991. - Pestic. Bioch. Physiol. 39: 291-301.*

MAJEVSKI M. S. 1991 - In: Frehse, H. (ed.). Pesticide Chemistry, VCh Weinheim, pp. 307-317.

PATERSON S., MACKAY D., TAM D. & SHIU W. Y. 1990. - Chemosphere 21/3: 297-331

SCHRÖDER P., LAMOUREUX G. L., RUSNESS D. G. & RENNENBERG H. 1990a. - Pestic. Chem. Physiol. 37: 211-218.

— , — & — . 1990b. - In: RENNENBERG H, BRUNOLD, C, DE KOK, L. J. & STULEN, I. (eds.) Sulfur Nutrition and Sulfur Assimilation in Higher Plants, SPB Academic Publishing, The Hague, pp. 245-248.

— , PFLUGMACHER S. & RENNENBERG H. 1992. - Angewandte Botanik. (in press).

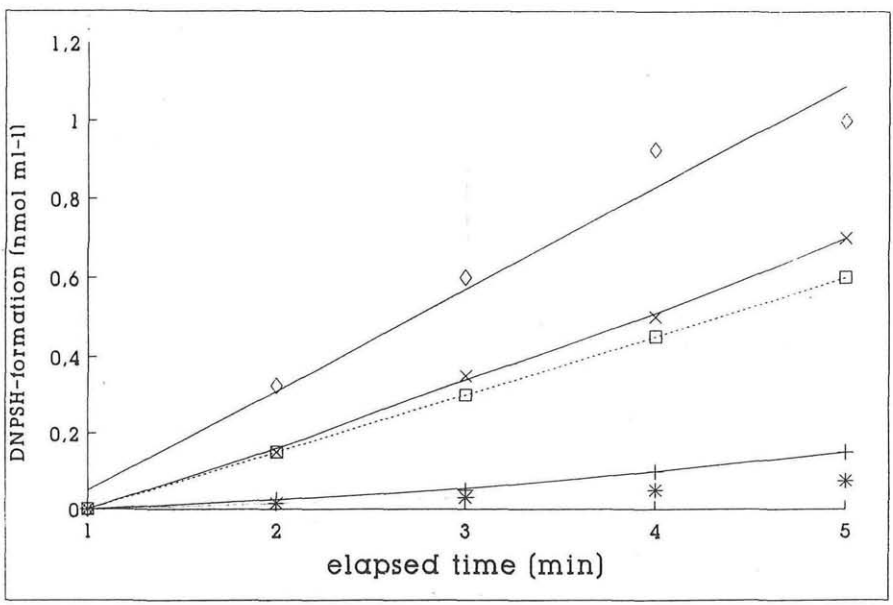

Fig. 1. CS-lyase activity extracted from spruce needle extracts. The protein extract (0.03 mg ml^{-1}) was incubated with 2,4-dinitrophenyl-S-cysteine and the formation of the 2,4-dinitrothiophenol was monitored spectrophotometrically. * Blank + DTT; + enzyme boiled; □ enzyme; × enzyme + DTT; ◊ enzyme doubled.

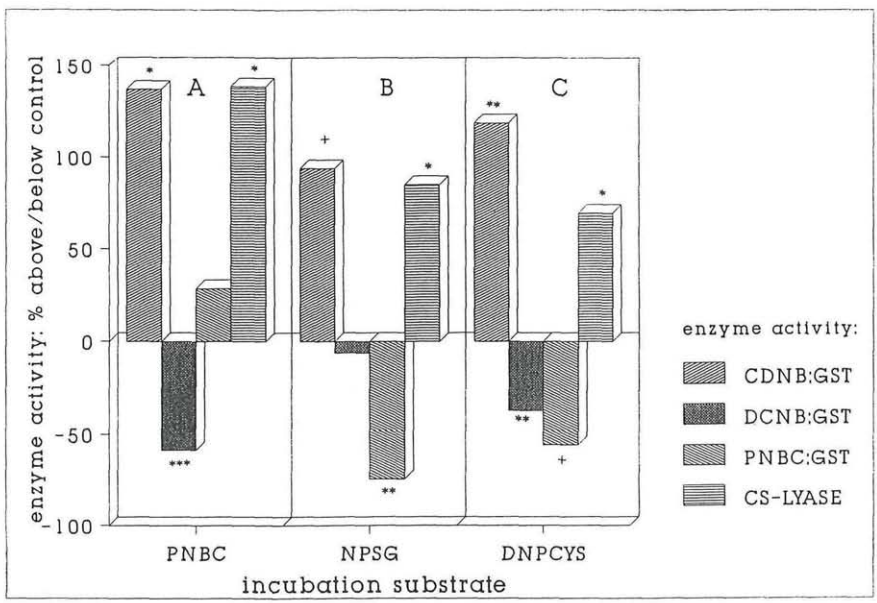

Fig. 2. Activities of GST and CS-lyase in spruce cuttings relative to untreated controls after 15 h incubation with several xenobiotics. Data are transformed from enzyme activities described in Table 1 and are means of 2 to 3 replicate experiments. Significance levels for the difference compared to controls are +, $p > 0.2$; *, $p > 0.1$; **, $p > 0.05$; ***, $p > 0.01$.