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Detoxification of Formaldehyde by the Spider Plant (*Chlorophytum comosum* L.) and by Soybean (*Glycine max* L.) Cell-Suspension Cultures[1]

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The phytotoxicity of formaldehyde for spider plants (*Chlorophytum comosum* L.), tobacco plants (*Nicotiana tabacum* L. cv Bel B and Bel W3), and soybean (*Glycine max* L.) cell-suspension cultures was found to be low enough to allow metabolic studies. Spider plant shoots were exposed to 7.1 μL L^{-1} (8.5 mg m^{-3}) gaseous [^{14}C]-formaldehyde over 24 h. Approximately 88% of the recovered radioactivity was plant associated and was found to be incorporated into organic acids, amino acids, free sugars, and lipids as well as cell-wall components. Similar results were obtained upon feeding [^{14}C]formaldehyde from aqueous solution to aseptic soybean cell-suspension cultures. Serine and phosphatidylcholine were identified as major metabolic products. Spider plant enzyme extracts contained two NAS^+-dependent formaldehyde dehydrogenase activities with molecular mass values of about 129 and 79 kD. Only the latter enzyme activity required glutathione as an obligatory second cofactor. It had an apparent K_m value of 30 μM for formaldehyde and an isoelectric point at pH 5.4. Total cell-free dehydrogenase activity corresponded to 13 μg formaldehyde oxidized h^{-1} g^{-1} leaf fresh weight. Glutathione-dependent formaldehyde dehydrogenases were also isolated from shoots and leaves of *Equisetum telmateia* and from cell-suspension cultures of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.). The results obtained are consistent with the concept of indoor air decontamination with common room plants such as the spider plant. Formaldehyde appears to be efficiently detoxified by oxidation and subsequent C1 metabolism.

Formaldehyde is a ubiquitous air pollutant that has been classified as a mutagen and suspected carcinogen (Senatskommission, 1992). Indoor air concentrations of formaldehyde are often higher than the legal German tolerance value of 100 nL L^{-1} (0.1 ppm, 120 μg m^{-3}) formaldehyde.[2] A screening program in German residential homes has revealed a concentration range of formaldehyde of between 12 and 260 nL L^{-1} (Umweltbundesamt, 1992). In an American survey, average values of around 50 nL L^{-1} formaldehyde for indoor sites and around 5 nL L^{-1} for outdoor sites were found (Shah and Singh, 1988). In special environments, such as mobile homes or energy-efficient houses, much higher values occur (Wolverton et al., 1984).

Formaldehyde was believed to be a central intermediate of photosynthetic carbon dioxide fixation (Strasburger et al., 1936; Florkin, 1977). In 1953 it was shown that carbon dioxide is directly assimilated by addition to ribulose-1,5-bisphosphate (Bassham et al., 1954). Nevertheless, [^{14}C]formaldehyde was well assimilated from aqueous solution by excised barley leaves (Krall and Tolbert, 1957). Nonenzymic adduct formation with Asn was detected in potato tissue (Mason et al., 1986). In addition, an FDH forming *S*-formylglutathione has been highly purified from pea seeds. This enzyme required NAD^+ and GSH as obligatory cofactors (Uotila and Koivusalo, 1979). A comparison of N-terminal sequences of published plant ADH gene sequences has revealed homology to the GSH-dependent FDH from *Escherichia coli* as well as from various animal species (Gutheil et al., 1992).

Chamber experiments have indicated that common room plants, in particular the spider plant (*Chlorophytum comosum* L.), greatly reduced the concentrations of gaseous formaldehyde and of other indoor air pollutants (Wolverton et al., 1984, 1989). The effect in part also occurred after removing the plant foliage, or with soil alone (Wolverton, 1984, 1989). The presumed detoxification of indoor air pollutants by room plants (Wolverton et al., 1984, 1989) has been widely publicized in the popular press and is also described in a recent book (Henseler, 1992). However, subsequent experiments indicated that a metabolic role was not involved, at least a distinctively light-phase metabolic one (Godish and Guindon, 1989; Godish, 1990). This negative result was consistent with the report that green plant leaves contain only negligible FDH activity, as tested with corn and spinach (Uotila and Koivusalo, 1983). None of the quoted contradictory whole plant studies employed radioactively labeled formaldehyde, so that mere adsorption could not be distinguished from metabolism. In view of the potential importance of indoor decontamination with room plants, spider plants have now been exposed to [^{14}C]formaldehyde, employing aseptically grown soybean (*Glycine max* L.) cell-suspension cultures in control experiments.

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[2] The conversion factor for formaldehyde is as follows: 1 ppm = 1 μL L^{-1} = 1.2 mg m^{-3} (Umweltbundesamt, 1992).

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Abbreviations: ADH, alcohol dehydrogenase; DEHP, diethylhexylphthalate; FDH, formaldehyde dehydrogenase.

MATERIALS AND METHODS

Chemicals

[^{14}C]Formaldehyde was purchased from Zinsser (Frankfurt, Germany; catalog No. CMM-160). All nonradioactive compounds were of analytical grade and were obtained commercially.

Plant Materials

Cell-suspension cultures of wheat (*Triticum aestivum* L. cv Heines Koga II), soybean (*Glycine max* L. cv. Mandarin), and maize (*Zea mays* L. cv Black Mexican Sweet) were grown in the dark as previously described (Sandermann et al., 1984; Komoßa et al., 1992). Adult plants of *Equisetum telmateia* were collected on a field site near Murnau (Bavaria). Spider plants (*Chlorophytum comosum* L. var variegatum) were grown in soil under normal office conditions using pots on a windowsill for the purpose of enzyme isolation.

For all other purposes, spider plants and tobacco (*Nicotiana tabacum* L. cv Bel W3 and Bel B) were cultivated in standard substrate/perlite (2:1) as described (Langebartels et al., 1991). Four days before treatment, spider plants (height of 10–15 cm) with several attached plantlets, or tobacco plants (height 15–20 cm) were placed for adaptation into the incubation hoods, made of plexiglass (ITE incubation hood, Infors), and kept at 21 ± 1°C. The incubation hood was installed below a lighting system (Philips TLD 18 W/25, 75 μE m^{-2} s^{-1}) with a 12-h photoperiod. The low light intensity used was comparable to typical office and indoor conditions.

Treatment with Formaldehyde

Intact Plants

General techniques for formaldehyde exposure were adopted from Green and Kulle (1986). For a large-scale fumigation experiment, an excised main spider plant shoot with four attached plantlets was placed into a desiccator (volume of 20 L) underneath the incubation hood. Total fresh weight of the shoots with leaves was 36.6 g. The total fresh weight of the aerial roots was an additional 12.7 g. During the entire experiment the base of the excised main stem was provided through a hose connection with water from outside the desiccator. The latter was connected to a trap system. The connection hoses between desiccator, trap system, and pump were made of viton. The plants were in contact with gaseous [^{14}C]formaldehyde over a period of 24 h (14 h in light). Throughout this time, RH was over 90%. At the end of the incubation time the chamber was flushed with pollutant-free air and remaining [^{14}C]formaldehyde and [^{14}C]carbon dioxide was absorbed during 3.5 h in absorption tubes (Kloskowski et al., 1981) containing 15 mL of sodium bisulfite (1%, w/v) (three tubes) and ethanolamine/ethylene glycol monomethylether (1:2, v/v) (two tubes). Gaseous [^{14}C]formaldehyde and $^{14}CO_2$ were absorbed to >95%.

Gaseous formaldehyde was prepared from aliquots of 37% (w/v) formaldehyde plus [^{14}C]formaldehyde stock solutions. Prior to evaporation, the solution containing 0.21 mg of [^{14}C]-formaldehyde (3.7×10^5 Bq) was heated to 110°C for 2 h in a sealed glass ampule to assure depolymerization. During the experiment, the air volume of the desiccator was constantly circulated by a ventilator to evaporate the [^{14}C]formaldehyde and to overcome the boundary-layer resistance of the leaves (wind speed >0.5 m s^{-1}). In the main experiment described, 20.9% of the initial radioactivity was still detected in the ampule used at the end of the experiment. Therefore, the amount of volatilized formaldehyde was 0.17 mg (2.9×10^5 Bq) in the 20-L volume of the desiccator used. This corresponded to a concentration of 7.1 μL L^{-1} (8.5 mg m^{-3}).

For phytotoxicity determination, nonradioactive formaldehyde was applied as above at calculated concentrations of 0.15, 1, or 10 μL L^{-1} for 5 h. Plexiglass chambers with 1 m^3 volume were used as described (Langebartels et al., 1991). Leaf damage was visibly assessed as percent damaged area. Amounts of gaseous formaldehyde were controlled semiquantitatively with Drger cartridges (catalog No. 6726760) and in some cases by GC.

Soybean Cell Cultures

[^{14}C]Formaldehyde was first heated as described above and was then added to the medium to a final aqueous concentration of 1 μL L^{-1} 40 mL^{-1} suspension (i.e. 33 μmol L^{-1}; total radioactivity of 3.7×10^4 Bq). The cells were incubated for 48 h at 27°C and 110 rpm in the dark. Control cultures were inactivated (20 min, 120°C) prior to incubation. At the end of the incubation time the culture flasks were aerated with sterile filtered air for 1 h. [^{14}C]Formaldehyde and [^{14}C]carbon dioxide were absorbed in aqua bidest and ethanolamine/ethylene glycol monomethylether as described above. For determination of phytotoxicity, the desired aliquot of nonradioactive formaldehyde in 10% (v/v) methanol was added in a total volume of 0.4 mL to the growth medium (40 mL) 2 d after inoculation. Cells were then incubated for 5 d.

Extracted and Separation of Metabolite Fractions

Leaves were homogenized in a mortar under liquid nitrogen and then extracted with a modified Bligh-Dyer mixture (methanol:dichloromethane:water, 2:1:0.8, v/v/v; 3.75 mL g^{-1} fresh weight) overnight at −25°C. The homogenates were filtered by suction and nonextractable residues were washed with the methanol:dichloromethane:water mixture. The dichloromethane and methanol:water phases were separated by adding 1.25 volumes of dichloromethane to the extract.

Extraction of the soybean cells and the culture medium was carried out as described (Sandermann et al., 1984; Harms and Langebartels, 1986). Phase separation was obtained by adding 0.5 volume of both aqua bidest and dichloromethane to the cell extract. The final residues left after repeated extraction were freeze dried and their radioactivity was determined after automatic combustion in a model 306 oxidizer (Packard, Frankfurt, Germany) followed by liquid scintillation counting of the [^{14}C]carbon dioxide formed. The radioactivity of the extracts was assayed by standard liquid scintillation counting procedures.

TLC was performed on precoated Silica Gel G 60 plates (Merck No. 5554) using the following solvent systems (parts by volume): (1) acetone:benzene:methanol:water (8:3:2:1;

Yoshida and Uemura, 1986); (2) chloroform:acetone:methanol: acetic acid:water (10:4:2:3:1; Yoshida and Uemura, 1986); (3) chloroform:methanol:17% ammonia (2:2:1; Brenner et al., 1967); (4) phenol:water (3:1, w/v; Brenner et al., 1967); (5) isobutyric acid:water:propanol-1:25% ammonia:propanol-2:butanol-1 (100:19:4:14:3:3; Komoßa et al., 1992). Radioactivity on the TLC plates was monitored by two-dimensional scanning with a TLC linear analyzer (LB 2842, Berthold, Wildbad, Germany). For the detection of lipids the plates were sprayed with 30% H_2SO_4 and heated or exposed to iodine vapor. Amino acids were located by spraying with ninhydrin:collidine reagent and heating at 110°c for 10 min. Spots were identified by comparison with the authentic reference compounds.

For TLC analysis, aliquots (5 mL) of the dichloromethane phases were concentrated by vacuum evaporation at below 40°C. After redissolving in a small amount of dichloromethane:methanol (1:2, v/v), lipids were separated by two-dimensional TLC in solvent systems 1 and 2 for the first and the second dimension, respectively. The application was performed under a stream of nitrogen. After concentrating an aliquot (5.5 mL) of the methanol:water phase of soybean cell extracts to a volume of 1 mL, the solution was fractionated into sugars, amino acids, organic acids, and phosphate esters by means of a combination of sulfopropyl- and diethyl[2-hydroxypropyl]aminoethyl-ion-exchange-Sephadex (Sigma, München, Germany) according to Redgwell (1980). The fraction of the amino acids was brought to dryness at below 30°C and dissolved in 1 mL of distilled water. Aliquots were applied to silica gel TLC plates and developed two dimensionally in solvent systems 3 and 4.

The radioactive nonextractable residues of soybean cells were analyzed by the sequential fractionation scheme of Langebartels and Harms (1985). After extraction with 20 mL of modified Bligh/Dyer mixture (see above), the residues were washed with 22 mL of methanol and afterwards with four 22-mL portions of distilled water. At each treatment step, the residues or wall material was sonicated in a sonic bath for 15 s and the remaining insoluble material was isolated on glass fiber filters. For the fractionation procedure, 80 mg of the wall material and 120 mg of added cellulose carrier was employed. Residues were enzymically treated with α-amylase and pronase E by the published procedures (Langebartels and Harms, 1985). The remaining material was further incubated with EGTA and then extracted with dioxane:water (9:1) and dioxane:2 N HCl (9:1). Following treatments with KOH (24%, w/v) and 72% (v/v) H_2SO_4 as described (Langebartels and Harms, 1985), the remaining insoluble material was combusted to CO_2 in an oxidizer and analyzed for radioactivity.

Enzyme Isolation and Characterization

Spider plant enzyme was prepared by modification of a method described by Uotila and Koivusalo (1974) for human liver. Freshly detached leaves of spider plants (100 g) were thoroughly homogenized under liquid nitrogen in a mortar with pestle. The powder was further homogenized in 100 mL of 50 mM Na_2HPO_4/KH_2PO_4 (pH 7.4), containing 5 mM DTE, about 100 mg of solid PMSF, and some sea sand. After filtration through a 50-μm nylon net and centrifugation for 30 min at 41,000g at 4°C, the precipitate was discarded. Solid ammonium sulfate was added to the supernatant to 45% saturation. The mixture was stirred at 4°C for 30 min and centrifuged at 41,000g for 30 min. The supernatant was brought to 75% saturation with solid ammonium sulfate and stirred at 4°C for 30 min. During ammonium sulfate fractionation the pH was maintained at 7.4 with 2 M NH_3. The precipitate was collected by centrifugation as above and dissolved in 2.5 mL of 50 mM Na_2HPO_4/KH_2PO_4 (pH 7.4), 5 mM DTE. Desalting was achieved on a Sephadex G-25 column (PD-10, Pharmacia) using 90 mM $Na_4P_2O_7$ (pH 8.0). The eluate was used for enzyme assays. It was stable for several weeks after addition of 30% (w/v) Suc and storage at −20°C. A similar work-up procedure was carried out with 70 g fresh weight of *E. telmateia* shoots and with stationary-phase cultured cells of wheat and maize. All reported enzyme activities refer to the deslated 40 to 75% ammonium sulfate protein fractions. Protein was determined by the Bio-Rad Protein Assay with albumin fraction V from bovine blood (Merck) as standard. The protein content of the eluate from chromatography columns was continuously monitored at 280 nm.

The assay mixture contained 90 mM $Na_4P_2O_7$ (pH 8.0), 1 mM GSH, 0.16 mM formaldehyde, and 1.5 mM NAD^+ (Uotila and Koivusalo, 1974). Where noted, the assay mixture also contained 3 mM pyrazole to block ADH activity type I. Formation of NADH was followed continuously over 3 min at 340 nm with a UV-160A spectrophotometer (Shimadzu) thermostated at 30°C. A blank cuvette without enzyme was always included. In addition, an incubation without GSH was performed to measure GSH-independent. FDH activity. Subtraction of this rate from the total rate gave an estimate of specific FDH. ADH type I activity was measured using 40 mM ethanol and 1.5 mM NAD^+ in 90 mM $Na_4P_2O_7$ (pH 8.0). Enzyme activity was calculated in terms of μmol min^{-1} mg^{-1} protein (Uotila and Koivusalo, 1974) by using a molar absorption value (340 nm) of 6300 (L mol^{-1} cm^{-1}) for NADH. The apparent K_m of formaldehyde was determined under the standard assay conditions using a Lineweaver-Burk plot.

Mol wts were determined on a Sephacryl S-200 column (2.6 × 61 cm). The column was equilibrated and eluted with 90 mM Tris-HCl (pH 7.4) containing 5 mM DTE. The standard proteins were located by their absorption at 280 nm. For chromatofocusing, the partially purified sample from the gel-filtration step was chromatographed on a Mono P HR 5/20 column (4 mL bed volume) using a fast protein liquid chromatography system (Pharmacia) according to the instructions of the manufacturer. Protein concentration was measured at A_{280}. Elution was with a pH gradient from pH 6 to 4 at a flow rate of 0.8 mL min^{-1}. Fractions of 0.5 mL were collected.

PAGE in the presence of SDS was performed with the PhastSystem (Pharmacia) using a calibrated 8 to 25% gradient as well as 12.5% homogeneous gels according to the manufacturer's instructions. Protein samples were denatured (10 min, 100°C) in a sample buffer containing 1% (w/v) SDS and 1% (v/v) 2-mercaptoethanol. The gels was silver stained. Polypeptide mol wt values were estimated by comparison with reference proteins (mol wt marker kit, Pharmacia).

RESULTS

Phytotoxicity of Formaldehyde

Spider plants were treated with 0.15, 1, or 10 μL L^{-1} gaseous nonradioactive formaldehyde in plexiglass incubation chambers and were then postcultivated for 1 week in pollutant-free air. The present study was restricted to short-term treatment (5 h). No visible foliar injury was observed at any of the concentrations used. Comparative studies were performed with ozone-sensitive and ozone-tolerant tobacco varieties that are routinely studied in this laboratory (Langebartels et al., 1991). Tobacco varieties Bel B (ozone tolerant) and Bel W3 (ozone sensitive) were both significantly damaged at 10 μL L^{-1} formaldehyde (>60% damaged leaf area). At 1 μL L^{-1} formaldehyde, the ozone-sensitive variety Bel W3 remained undamaged, whereas Bel B showed small lesions that later developed into small, brownish, necrotic spots (10% damaged leaf area). In contrast, ozone (0.15 μL L^{-1}) caused visible lesions only on Bel W3 but not on Bel B (cf. Langebartels et al., 1991). Both formaldehyde and ozone symptoms were detectable mainly on middle-aged leaves. The dependence on leaf age indicated strong developmental control of damage, middle-aged leaves generally having the highest stomatal conductance (Langebartels et al., 1991). Spider plants in these experiments were not visibly damaged at 0.15 μL L^{-1} ozone (5 h) and showed only initial damage (turgor loss, Chl bleaching, with later partial necrosis) at 1 μL L^{-1} ozone. Spider plants thus seemed to be rather resistant to formaldehyde as well as ozone.

In separate studies, formaldehyde was added to the growth medium of soybean suspension cultures in concentrations of 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M, followed by incubation for 5 d. Only the highest formaldehyde concentration resulted in decreased cell dry weight (by 48%). The cell dry weight of untreated cells (365 ± 8 mg) decreased to 188 ± 13 mg at 1 mM formaldehyde. In addition, the conductivity of the culture medium increased from 0.34 ± 0.04 (control) to 1.9 ± 0.07 mS cm^{-1}.

Formaldehyde Uptake and Metabolism by Spider Plants

Gaseous nonradioactive formaldehyde (5–7 μL L^{-1}) was removed by spider plants from the atmosphere of the plexiglass chamber within 5 h to below the detection limit (0.2 μL L^{-1}) of the Dräger formaldehyde monitors. Control experiments using pots containing humid soil without plants showed a linear formaldehyde decrease to 3 μL L^{-1} in 5 h. Soil adsorption effects of formaldehyde have been described previously (Wolverton et al., 1984, 1989; Godish and Guindon, 1989; Godish, 1990).

A large-scale uptake study was carried out with [^{14}C]-formaldehyde (7.1 μL L^{-1}) using a detached spider plant main shoot with four associated plantlets. The total incubation time was 24 h, of which 10 h were in the dark. At the end of the incubation period, the remaining formaldehyde in the gas phase was trapped in a trapping system (4.8% of initial radiolabel). The total recovery was low (56.4%), probably due to adsorption effects. Nevertheless, about 88% of the recovered radioactivity was found to be plant associated (Table I). The individual incubated plantlet shoots as well as the pooled aerial roots were extracted with methanol:dichloromethane:water (2:1:0.8). Radioactivity was found in the soluble extracts and also in the bound residues as summarized in Table I. The pooled total values were 33.9% for the soluble extract and 11.7% for the bound fraction (Table I). After phase separation of the individual plant extracts, about 90% of the radioactivity was found in the methanol:water phases and 10% was found in the dichloromethane phases (Table I). This distribution was similar in the shoots and roots of the individual plantlets.

Table I. *Distribution of ^{14}C (percent of applied radioactivity) after incubation of a spider plant shoot and associated plantlets with [^{14}C]formaldehyde*

Overall Distribution of Radioactivity	
Combined fractions	Percent of applied radioactivity
Methanol:water phase of soluble extract	33.9
Dichloromethane phase	4.2
Bound residues	11.7
Total plant associated	49.9
^{14}C in rinsing fluids	1.8
^{14}C in trapping system	4.8
Total recovery, percent of initial	56.4

^{14}C Distributions in Individual Samples					
	Plantlet number				Combined aerial roots
	1	2	3	4	
Fresh weight (g)	10.5	7.2	10.5	8.4	12.7
	% of applied radioactivity				
Total soluble extract	9.0	8.1	9.1	6.3	5.6
Methanol:water phase	8.1	7.2	8.0	5.6	5.1
Dichloromethane phase	0.9	0.9	1.1	0.7	0.5
Bound residues	2.1	2.0	2.1	1.3	4.3
Total recovery	11.1	10.1	11.2	7.6	9.9

The radioactivity in the combined methanol:water phases shown in Table I was separated into four classes of natural compounds according to Redgwell (1980) (Table II). Most of the radioactivity was found in the sugar fraction and in organic acids. The dichloromethane phases of the spider plant extracts were examined by two-dimensional TLC (solvent systems 1 and 2). More than 10 radioactive spots were detected by radioscanning. All these spots were positive with iodine vapor and a sulfuric acid spray. The major incorporation products co-chromatographed with phosphatidylcholine (R_F 0.05 in the first and R_F 0.13 in the second dimension) and with steroids or other nonpolar lipids (R_F 0.7 in the first and R_F 0.87 in the second dimension).

Formaldehyde Assimilation in Soybean Suspension Cultures

Using a standard protocol for xenobiotic chemicals (Sandermann et al., 1984; Harms and Langebartels, 1986), soybean suspension cultures in logarithmic growth phase were incubated with 1 μL L^{-1} [^{14}C]formaldehyde (3.7×10^4 Bq) for 48 h. Thirty-four percent of the applied radioactivity was taken up into the cells. The label (32%) found in the culture

Table II. *Distribution of the ^{14}C radioactivity in the aqueous phases of spider plantlets (see Table I) and of soybean cells (see Table III) after extraction by the Bligh/Dyer procedure*

The combined aqueous phases were separated into the four classes of sugars, amino acids, organic acids, and phosphate esters according to Redgwell (1980).

Fraction	^{14}C Radioactivity	
	Spider plantlets	Soybean cells
	%	
Amino acids	0.6	42.9
Organic acids	11.6	31.4
Sugars	84.6	19.7
Phosphate esters	1.3	5.9
Recovery	98.1	99.9

Table III. *Incubation of cell-suspension cultures of soybean with [^{14}C]formaldehyde (1 $\mu L\ L^{-1}$; 3.7 × 10^4 Bq)*

Average values from two parallel experiments are shown.

Fraction	Percent of Applied Radioactivity	
	Native cultures	Autoclaved cultures
Cells	34	4.7
Methanol:water phase	10.6	0.4
Dichloromethane phase	7.4	0.5
Bound residues	16.0	3.8
Medium	31.9	93.2
Methanol:water phase	28.7	83.9
Dichloromethane phase	3.2	9.3
Volatilized	6.1	2.7
Total recovery	72.0	100.6

medium was not characterized. The methanol:water phase of the native soybean cells was separated into four classes of organic compounds by the method of Redgwell (1980). The ^{14}C label was predominantly found in amino acids and organic acids (Table II). The autoclaved control cells contained only a small part (4.7%) of radiolabel within the cells. Ninety-three percent was present in the medium and the recovery was nearly 100% (Table III). The labeled materials of the control experiment were not characterized.

One-dimensional TLC (solvent system 5) of the isolated cellular fractions gave the following results: no free formaldehyde was detected in the soluble fractions of the soybean cells. Radiolabel in the organic acids was present in a large spot with a R_F 0 to 0.4, tailing up to R_F 0.6. The main spot seemed to comprise glycolate as well as glycerate (solvent system 5). Radioactivity in the amino acid fraction was present to 90% as a single prominent spot of R_F 0.6 to 0.8 (position of Ser); 10% was located at R_F 0.4 (solvent system 5). The sugar fraction comprised several spots in this solvent system, whereas activity in the phosphoester fraction was too low to allow good resolution. The amino acid fraction was further analyzed by two-dimensional TLC (solvent systems 3 and 4). Again, more than 90% of the label was found in one, distinct, radioactive spot co-chromatographing precisely with the Ser standard. The more prominent of the minor radioactive spots co-chromatographed with the Phe standard.

The dichloromethane phase after extraction of the incubated soybean cells was analyzed for lipid components. Five spots were detected by two-dimensional TLC (solvent systems 1 and 2). Fifty percent of the radioactivity in this phase co-migrated with phosphatidylcholine (R_F 0.05 in the first dimension, R_F 0.5 in the second dimension). A spot comprising 28% of the label migrated with steroids and other unidentified nonpolar lipid compounds (R_F 0.6 in the first dimension, R_F 0.95 in the second dimension).

The bound residues in soybean cells contained 16% of the applied radioactivity and were analyzed by the sequential cell-wall fractionation scheme of Langebartels and Harms (1985). This procedure determines the distribution of radiolabel between operationally defined cell-wall fractions such as protein, pectin, hemicellulose, and lignin. As shown in Table IV, most of the label was found in the pronase E-solubilized fraction (protein) and the dioxane:H_2O and dioxane:HCl fractions (lignin). Low or nondetectable labels were found in the KOH soluble (hemicellulose), the sulfuric acid hydrolysate (cellulose), and the final residue.

Enzyme Isolation and Characterization

A soluble enzyme extract was prepared from spider plant leaves by standard methods. After ammonium sulfate precipitation, two peaks of FDH activity could be detected by gel-permeation chromatography (Fig. 1). By comparison with reference proteins, molecular mass values of about 126 and 79 kD could be assigned. The latter activity depended on the presence of GSH in the assay mixture. SDS gel electrophoresis of the peak fraction revealed a prominent protein band of 42 kD, consistent with but not proving a dimer structure. The specific enzyme activities were between 2 and 6 for the rather variable GSH-independent and at 12.8 nmol min^{-1} mg^{-1} protein for the much more constant GSH-dependent enzyme activity. A total value of 15 nmol min^{-1} mg^{-1} protein corresponded to 7.1 nmol formaldehyde min^{-1} g^{-1} leaf fresh weight. This in turn corresponds to 12.8 μg formaldehyde h^{-1} g^{-1} leaf fresh weight. The isoelectric point of the GSH-

Table IV. *Sequential solubilization of ^{14}C from cell-wall fractions (the bound residue) derived from [^{14}C]formaldehyde-treated soybean cell-suspension cultures (see Table III)*

Mean values from two experiments are given. The presumed nature of the operationally defined plant component is given in parentheses.

Solubilizing Agent (Plant Component)	Amount of Initial ^{14}C
	%
α-Amylase (starch)	5
Pronase E (proteins)	37
EGTA (pectins)	19
Dioxane:H_2O and dioxane:HCl (lignins)	40
KOH (hemicelluloses)	3
H_2SO_4 (cellulose)	0
Final residue (recalcitrant)	3
Total	107

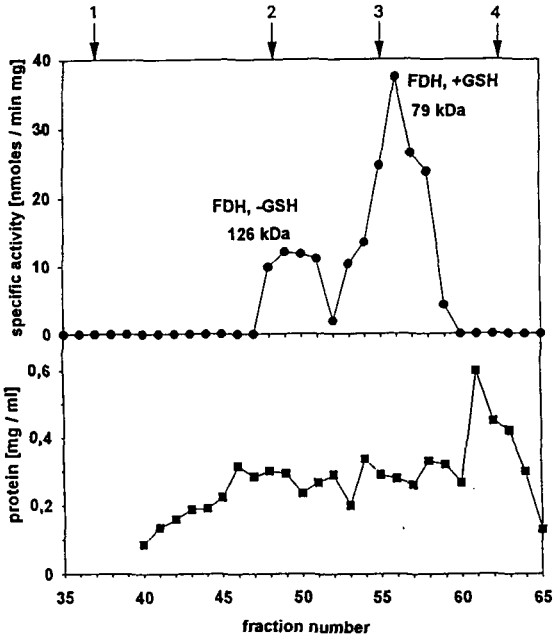

Figure 1. Molecular mass determination of spider plant FDH. The GSH-dependent (+GSH) and the GSH-independent (−GSH) FDH activities were separated on Sephacryl S-200 (upper). The column was calibrated with the following protein markers whose positions are indicated by arrows. 1, Blue dextran, 2000 kD; 2, yeast ADH, 154 kD; 3, transferrin, 80 kD; 4, ovalbumin, 45 kD. The flow rate was 0.2 mL min^{-1}, fractions of 2.5 mL were collected. The fractions were assayed with 0.16 mM formaldehyde, 1.5 mM NAD^+, and 3 mM pyrrazole with 1 mM or without GSH. The protein elution curve is shown in the lower panel.

dependent FDH activity was determined by chromatofocusing (Fig. 2). An isoelectric point of 5.45 was determined. The apparent K_m value was determined to be 30 μM under the conditions of the standard assay. It should be noted that S-hydroxymethyl-GSH rather than formaldehyde itself is the true substrate (Uotila and Koivusalo, 1983).

DISCUSSION

Phytotoxicity

The present results on the phytotoxicity of formaldehyde are consistent with the limited literature reports on other plant species. Several plant species remained unchanged after 2 or 5 h exposure to up to 2 μL L^{-1} formaldehyde (Haagen-Smit et al., 1952). Systematic longer-term studies (2 weeks) have demonstrated necrotic effects with various plant species, although growth reductions without visible symptoms were caused at much lower formaldehyde concentrations. Tobacco Bel W3 was significantly affected by about 1 μL L^{-1} formaldehyde (van Haut et al., 1979). In the present study, tobacco Bel B was more strongly damaged by formaldehyde than Bel W3, which was opposite to the ozone sensitivity of these tobacco arieties. Both varieties were strongly damaged at 10 μL L^{-1} formaldehyde. Sensitivity to both gases seemed to depend on stomatal conductance because both conductance and damage were highest in leaves 3 and 4 from the top of the tobacco plants. In a comparative tes of various organic air pollutants, formaldehyde was, after ethylene, the second moist powerful toxicant (van Haut et al., 1979). A yearly average concentration of 16 nL L^{-1} formaldehyde was suggested as tolerance value based mainly on growth reduction effects (van Haut et al., 1979). Actual environmental concentrations are often higher.

Wole Plant Metabolism

Spider plant shoots are shown here to be able to take up and extensively metabolize gaseous formaldehyde. Practically all of the plant-associated radioactivity consisted of metabolites. A leaf fresh weight of 36.6 g and an initial total amount of formaldehyde of 170 μg were used. In view of the total recovery of only 49.9% (Table I), an approximate amount of 80 μg of formaldehyde was converted to metabolites. The approximate minimum in vivo rate was 80 μg 36.6 g^{-1} leaf fresh weight 24 h^{-1}, corresponding to 0.1 μg g^{-1} fresh weight h^{-1}. However, no evidence was as yet obtained to establish a linear relationship between formaldehyde uptake and time. Metabolism was measured under hydroponic conditions so that effects of soil microorganisms could be excluded. The control experiments with aseptically grown soybean cell cultures agreed in principle with the results obtained with spider plants, so that microbial metabolism was also ruled out as an explanation. Many metabolic products, such as organic acids, sugars, amino acids, and insoluble residues were labeled. This is consistent with dispersion of the ^{14}C label through general C1 metabolism.

Ser and phosphatidylcholine were highly labeled. These compounds are known to be directly derived from C1 metab-

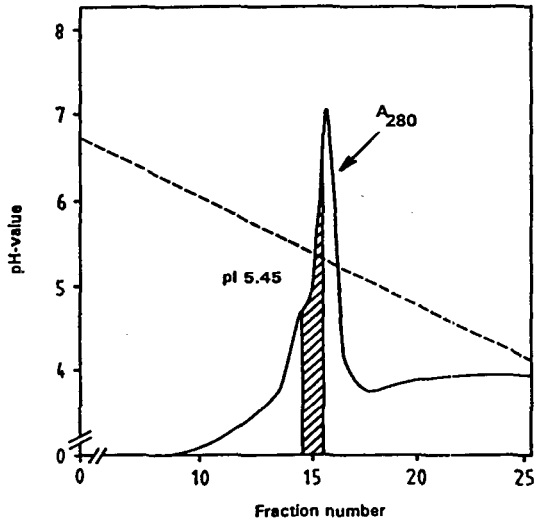

Figure 2. Isoelectric point of the GSH-dependent FDH (chromatofocusing). FDH activity was assayed with 0.16 mM formaldehyde, 1.0 mM GSH, 1.5 mM NAD^+, and 3.0 mM pyrrazole (3 min, 30°C). Only fraction No. 15 (hatched area) contained FDH activity. The protein content of the eluate was continuously monitored at 280 nm (|), as was the pH value (- - -).

olism with participation of methylene tetrahydrofolate and S-adenosylmethionine, respectively. The prominent labeling of lignin (Table IV) could be due to the high content of methoxy groups in lignins. Part of the labeling may be due to addition reactions of formaldehyde to NH_2 and sulfhydryl groups, as documented by the study in potato tissue (Mason et al., 1986). However, additional reactions to endogenous nucleophiles have not been differentiated in the present study. The metabolite pattern found here is in agreement with that found in previous studies where [^{14}C]formaldehyde was administered to barley leaves from aqueous solution (Krall and Tolbert, 1957) or where it was administered to sunflower plants from the gas phase at a concentration of 20 $\mu g\ m^{-3}$ (Girard et al., 1989). The present and the previous studies using [^{14}C]formaldehyde remove an important gap in the NASA studies (Wolverton et al., 1984, 1989), where adsorption phenomena could not be differentiated from true metabolism. It is not clear why formaldehyde metabolism was not observed in studies (Godish and Guindon, 1989; Godish, 1990) designed to reexamine the NASA whole-plant studies.

Metabolic Enzymes

A high metabolic potential of spider plants is indicated by the two plant FDH activities now discovered. The GSH-dependent enzyme may on the basis of mol wt, K_m value, and isoelectric point be identical to the FDH (EC 1.2.1.1) previously purified to homogeneity from pea seeds (Uotila and Koivusalo, 1979). This GSH-dependent enzyme consisted of two 42-kD subunits. The GSH-dependent FDH has remained rather constant during evolution (Uotila and Koivusalo, 1983; Gutheil et al., 1992), thus providing another example for the "green liver" concept of plant xenobiotic metabolism (Sandermann, 1992). Gutheil et al. (1992) mentioned the close homology of the *Adh1* and *Adh2* genes of maize to the GSH-dependent FDH. However, recent results indicate that these enzymes are not identical (J. Fliegmann and H. Sandermann, unpublished results). Other plant protein extracts isolated in this laboratory were tested for GSH-dependent FDH activity with the following results (U. Wippermann, unpublished results). A soluble protein extract from *E. telmateia* stems and leaves had a specific activity of 17 nmol $min^{-1}\ mg^{-1}$ protein. GSH-dependent FDH activity (13 nmol $min^{-1}\ mg^{-1}$ protein) was also detected in the extracts from cultured wheat cells. The protein extract from cultured maize cells had a specific activity of 10 nmol $min^{-1}\ mg^{-1}$ protein. Gel-permeation chromatography in analogy to Figure 1 revealed a similar molecular mass value (about 82 kD) as was found for the spider plant enzyme. The isoelectric point under the conditions of Figure 2 was at pH 5.9. The apparent K_m value of the GSH-dependent maize enzyme was 10 μM (at constant 1.5 mM NAD^+).

The specific activity of the spider plant enzyme (15 nmol $min^{-1}\ mg^{-1}$ protein) corresponded to 12.8 μg formaldehyde $h^{-1}\ g^{-1}$ fresh leaf weight. This figure is more than 100-fold higher than the present in vivo metabolic rate (see above). This indicates that the formaldehyde-detoxifying system in the spider plantlets was far from being saturated in the main experiment of Table I. Remarkably, the theoretical enzyme capacity determined here for spider plants is of the same order of magnitude as that previously determined for intact barley shoots (Krall and Tolbert, 1957; 5 $\mu g\ h^{-1}\ g^{-1}$ fresh weight). On the basis of enzyme capacity, a single 300-g spider plant could in 6 h detoxify a 100 m^3 room contaminated with 0.2 $\mu L\ L^{-1}$ formaldehyde. This calculation assumes V_{max} conditions and neglects the likelihood of a limitation by stomatal conductance. Nevertheless, the calculation lends support to the basic idea of room decontamination with plants as published in the NASA studies (Wolverton et al., 1984, 1989). The previous report of only negligible FDH activity in plant leaves (Uotila and Koivusalo, 1983) is probably due to the presence of inhibitors that were removed by the enrichment procedures employed in the present study. Quantitative experiments with various intact plants and with [^{14}C]formaldehyde under simulated indoor conditions should be carried out to precisely define stomatal conductance and enzyme capacity for detoxification. Such experiments should clarify the extent of decontamination through plant metabolism under real indoor and outdoor conditions. A further need for additional experiments comes from the proposal that formaldehyde metabolism is involved in the mode of action f field-sprayed methanol that has led to spectacular increases in crop yields (Nonomura and Benson, 1992).

Plants as Sinks for Organic Air Pollutants

There are few literature reports documenting the uptake and metabolism of gaseous xenobiotics by plants. Generally, plant uptake of gaseous or aerosol-bound xenobiotics occurs through stomata or cuticles. Further permeation into the tissue can then result in metabolism. The basic processes with their overall rate constants are summarized in Figure 3. Several studies are available for illustration. For example, tetrachloroethene showed a nearly reversible deposition onto spruce needles with only a low rate of metabolism (Figge, 1990). In contrast, uptake of furfural by spruce needles was practically irreversible (Figge, 1990). Furfural appeared to be

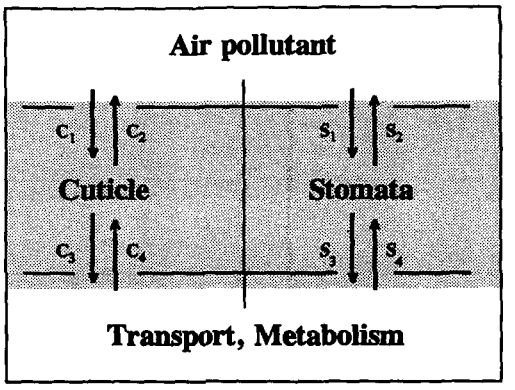

Figure 3. Plants as sinks for organic air pollutants. In the scheme shown, lowercase letters symbolize overall rate constants. Organic air pollutants may be taken up or released by cuticles (c_1 and c_2) or by stomata (s_1 and s_2). In both cases, uptake may be followed by transport and metabolism within the leaf tissue (c_3 and s_3) or by release (c_4 and s_4).

taken up through the stomata and then to be rapidly metabolized. Remarkably, the metabolite patterns of tetrachloroethene as well as furfural were closely similar in spite of their great difference in physical behavior (I. Gennity and H. Sandermann, unpublished results). Free or aerosol-bound nonpolar xenobiotics are generally likely to be taken up into the plant cuticle compartment. The subsequent transport and metabolism rate (c_3 of Fig. 3) may be low, but will under steady-state conditions be above zero even for very nonpolar xenobiotics (Riederer and Schönherr, 1989). Permeation may be facilitated by intracellular plant metabolic enzymes that have been shown to exist even for lipophilic xenobiotics such as DDT (Arjmand and Sandermann, 1985; H. Sandermann, unpublished data), hexachlorobenzene (Sandermann et al., 1984), and DEHP (Krell and Sandermann, 1985). In the case of DEHP metabolism it was shown that addition of isolated cuticles to the degradative enzyme assay led to strong inhibition, probably by back-partitioning (Krell and Sandermann, 1985; c_4 of Fig. 3). With regard to the stomatal pathway, high amounts of CO_2 (up to 140 mg g^{-1} dry weight h^{-1}) are known to be taken up and metabolized by plants (Larcher, 1983). Conceptually, rate constant s_3 may be dominant for CO_2 as well as furfural. The present results on formaldehyde are also consistent with a high relative value of s_3. In three cases (pentachlorophenol, 3,4-dichloroaniline, and DEHP) metabolic in vivo rates of cultured cells have been shown to be close to the in vitro activities of the initial metabolic enzymes (Komoßa et al., 1994). This result was probably due to the absence of cuticles or stomata in cultured cells. All quoted results remain as yet rather isolated.

Much more systematic studies are required to arrive at predictions on the sink activity of plant species for xenobiotics with different physical properties. As shown by the example of carbonylsulfide (Brown and Bell, 1986), the metabolic sink function of plants is likely to be of importance for the global fate of many trace contaminants.

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