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High-performance size-exclusion chromatographic characterization of water-soluble polymeric substances produced by *Phanerochaete chrysosporium* from free and wheat cell wall bound 3,4-dichloroaniline

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Abstract

A high-performance size-exclusion chromatographic (HPSEC) method was developed and optimized for the separation of aq. soluble polymeric xenobiotics. The use of this method for the characterization of aq. soluble polymeric substances produced by *Phanerochaete chrysosporium* cultures from [*ring*-U-^{14}C]-3,4-dichloroaniline ([^{14}C]DCA) and wheat cell wall bound [*ring*-U-^{14}C]-3,4-dichloroaniline was demonstrated by on-line UV detection at 280 nm and radioactivity tracing. The polymeric nature of aq. soluble lignin-like ^{14}C-products was shown by polydispersity and correspondence of the UV signal at 280 nm with the radioactivity trace. The higher polydispersity and molecular mass of lignin-like substances in low-C cultures as compared to those in low-N cultures were found to be associated with lower mineralization rates. Results indicated that the major portion of copolymerized DCA was apparently covalently bound via labile bonds to the α-C atom of the lignin monomer side chains on native wheat cell wall.

1. Introduction

Lignin has been regarded by many scientists as plant's major metabolic sink for the physiological removal of various types of pesticidal xenobiotics from plant intracellular systems via copolymerization. It was reported, for example, that ca. 60–80% of applied 3,4-dichloroaniline could be copolymerized into lignin by wheat plants [1]. As this biopolymer constitutes about 15–25% of the organic substances in some plants [2], it could be considered a major vehicle for the accumulation of diverse types of pesticidal xenobiotics in the natural environment [1]. The biopolymer lignin is formed by cross-polymerization of phenylpropane units via carbon–carbon and ether bonds, and is covalently bound to cell-wall polysaccharides (lignin–carbohydrate complexes, LCC) through hydrogen and ether bonding [3]. The herbicidal xenobiotic 3,4-dichloroaniline (DCA) is shown to be linked to the α-carbon of the phenylpropane side chain of lignin monomers during incorporation into plant lignin [1]. Until now, this group of copolymers has been successfully analysed by ^{1}H NMR spectroscopy, and its molecular mass distribution, e.g. for DCA–lignin, was successfully determined by Sephadex LH-60 chromatography

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[1,4]. In the past, lignin degradation products were analysed by gas chromatography, reversed-phase high-performance liquid chromatography, whereas lignin and lignin–cellulosic substances were characterized by Fourier transform infrared (FTIR) spectrometry, ^{13}C NMR, and pyrolysis gas chromatography–mass spectrometry (Py-GC–MS) [2,3,5]. These methods provide information on the phenolic monomeric constituents of lignin, allowing chemical characterization and significant comparison of treated and untreated materials, but they give no information on the molecular mass distribution of native lignin, oligomers derived from lignin degradation of lignin–carbohydrate complexes [3]. Molecular mass distributions of industrial lignin and lignocellulosics of wheat straw have been investigated by size-exclusion chromatography (SEC) and high-performance size-exclusion chromatography (HPSEC) [3], but HPSEC of lignin–pesticidal complexes for environmental studies, e.g. biodegradation and bioavailability, has not been performed to a great extent. The ubiquitous white rot fungus, *Phanerochaete chrysosporium*, plays an important role in the biodegradation, mineralization and bioavailability of free and lignin-bound pesticides, e.g. chloroanilines, polychlorinated hydrocarbons [6], in agricultural and forest ecosystems. The unique feature of *Phanerochaete chrysosporium* to mineralize a diverse range of xenobiotics, especially in low-N culture media, has been well documented. However, there are controversies about the role of its lignin-peroxidase in mineralization, polymerization or depolymerization of degradation products during bioconversion of free and bound xenobiotics [6].

The intent of the present paper is to describe a newly developed HPSEC method and to demonstrate its applicability to the characterization of aq. soluble polymeric substances produced by the white rot fungus *Phanerochaete chrysosporium* from [*ring*-U-^{14}C]-3,4-dichloroaniline and wheat cell wall [*ring*-U-^{14}C]-3,4-dichloroaniline–lignin metabolite fractions. Such a research is essential not only for the investigation of the ecotoxicological relevance of free and bound pesticidal residues in the environment (mineralization, release, bioavailability), but also for the biological detoxification of contaminated soils or other materials.

2. Experimental

2.1. Chemicals

All chemicals (p.a. grade) and solvents (HPLC grade) used in our studies were obtained either from Riedel-de Haën (Germany) or from Merck (Germany). Water used for the mobile phase of the HPSEC system was four times demineralized using Seralpur pro 90C system (Seral, Ransbach-Baumbach, Germany). The molecular mass calibration standards with peak molecular mass, M_p 1500, 5400, 8000, 19 300, 46 000, 73 500, 95 600, 200 000, 400 000, 780 000 (polystyrolsulphonate Na-salt; Polymer Science Labs., USA) were obtained from Macherey-Nagel (Germany). Blue Dextran (approx. molecular mass $2 \cdot 10^6$) was purchased from Sigma (Germany). Sitka spruce (*Picea sitchensis* L.) milled wood lignin (spruce in situ lignin) standard was prepared according to Lapierre et al. [7].

2.2. HPSEC instrumentation

A Merck-Hitachi HPLC system (L-6200 intelligent pump, Merck, Germany) was equipped with a Rheodyne injector Model 7125, an UV detector (Model 757 absorbance detector, Applied Biosystems, USA), and a HPLC radioactivity monitor (LB 505, Berthold, Germany). It was connected to a HPSEC (size exclusion) column GFC 300-8 with hydrophilic gel surfaces (column dimension, 300 × 7.7 mm I.D.; particle size, 8 μm; pore size, 300 Å; Macherey-Nagel, Germany) with a pre-column GFC 8P (50 × 7.7 mm I.D.) packed with the same gel as used for the main column.

The UV and radioactivity signals were acquired and processed by a PC-based software system Ramona (Raytest, Germany) and a Merck-Hitachi Integrator (D-2500 Chromato-Integrator).

2.3. Chromatographic conditions for HPSEC

During development of the HPSEC system, the effects of Na_2HPO_4 buffer (molarity, pH), $NaNO_3$ (molarity), and the organic additive methanol on the hydrophobic interactions as well as on the separation were studied. The flow-rate of the mobile phase for HPSEC separation was optimized. The optimized system used the following solvent system as mobile phase: 5 m*M* Na_2HPO_4 + 0.1% MeOH (pH 7.0) at a flow-rate of 0.5 ml/min. Samples were dissolved either in 5 m*M* Na_2HPO_4 + 0.1% MeOH, pH 7.0 (soluble products), or in 0.1 *M* Na_2HPO_4, pH 11.18 (polystyrolsulphonate Na-salt standards; Sitka spruce milled wood lignin). The pH of the mobile phase was adjusted using 3 *M* H_3PO_4, and the mobile phase was filtered through 0.2-μm filters (filter type RC 58, Schleicher and Schuell, Germany). Usually 20-μl samples were injected, however, radioactivity traces of the HPSEC analysis of DCA–lignin degradation products were obtained with an injection volume of 100–500 μl. Blue dextran (9.32 μg) and KNO_3 (1% aq. solution) were injected at a volume of 5 μl.

The UV signals were detected at 280 nm at high detector sensitivity (0.01 AUFS) for 20-μl injection volumes and at a low-detector sensitivity (2.0 AUFS) for 100-μl injection volumes. The elution of KNO_3 was monitored at 260 nm, and that of blue dextran at 380 nm.

2.4. Reversed-phase high-performance liquid chromatography

Reversed-phase high-performance liquid chromatography (RP-HPLC) of aq. soluble lignin fractions was performed with a gradient of 89.4% CH_3CN + 0.6% MeOH in H_2O (solvent B) and H_2O (solvent A) on a narrow bore column (250 × 4 mm I.D., 3 μm particle size, stationary phase Spherisorb C_8, flow-rate 0.6 ml/min) as follows: 0 min 30%, 5 min 40%, 10 min 45%, 15 min 50%, 18 min 55%, 20 min 60%, 25 min 75%, 27 min 100%, 29 min 100%, and 30 min 30% B in A. The column was calibrated using the following synthetic, authentic standards: N-(3,4-dichlorophenyl)glucoside (t_R 7.5 min), N-(3,4-dichlorophenyl)succinamide (t_R 15.2 min), N-(3,4-dichlorophenyl)succinimide (t_R 19.0 min), and 3,4-dichloroaniline (t_R 23.7 min) [8].

The detection of UV signals (250 nm, Model 757 absorbance detector, Applied Science, USA) and radioactivity signals (Model Berthold LB 505, Germany) was performed on-line as described for the HPSEC set-up.

2.5. Fermentation conditions

Phanerochaete chrysosporium cultures were maintained in the low-C and low-N media according to Kirk et al. [9] for 21 days, with veratryl alcohol added to the culture media for the stimulation of ligninase production. The concentration of DCA (non-labelled and/or ^{14}C-labelled DCA) was adjusted to 50 μmole/40 ml culture medium, with the radioactivity of [^{14}C]DCA in the treatment group "free DCA + bound [^{14}C]DCA" being half (0.169 μCi) that of the radioactivity applied for the treatment groups "free or bound [^{14}C]DCA" (0.338 μCi). The details of the fermentation conditions will be published elsewhere [10].

2.6. Preparation of wheat cell wall [^{14}C]DCA–lignin metabolite fractions

A wheat cell wall [^{14}C]DCA–lignin metabolite fraction was prepared by pooling the thoroughly extracted insoluble wheat residues from parts of the plant (*Triticum aestivum* var. Goetz) with the highest incorporation of ^{14}C [11]. The final dried wheat cell wall [^{14}C]DCA–lignin metabolite fractions had a consistency of irregular-sized light-brown strips, and showed a specific radioactivity of 5.14 μCi/g.

2.7. Water-soluble wheat cell wall [^{14}C]DCA–lignin degradation products produced by Phanerochaete chrysosporium

The water-soluble wheat cell wall [^{14}C]DCA–lignin degradation products were pooled from low-C and low-N culture filtrates of *Phanero-*

chaete chrysosporium at the end of the fermentation period (21 days) following exhaustive extraction with ethylacetate and *n*-butanol at pH 8.0 and 2.5, and subsequent adjustment of the pH of the unextractable aq. phase to 7.0 [10]. After evaporation in vacuo, the residues from the unextractable aq. phase (pH 7.0) were dissolved in appropriate volumes of 5 m*M* Na_2HPO_4 + 0.1% MeOH (pH 7.0) and analysed as described above.

2.8. Calibration of the HPSEC column by molecular mass (M_p) standards and estimation of molecular mass

The calibration curves of log M_p vs. elution volume, V_e, (see sections 2.1 and 2.3) were generated by Sigma-Plot software (Jandel, USA). The void volume (V_0) was determined by polystyrolsulphonate Na-salt at M_p 780 000 and blue dextran at an M_p of approx. $2 \cdot 10^6$, and the total permeation volume (V_p) was determined by KNO_3 (M_r 102).

The calibration curves of log M_p vs. elution volume (V_e) were used for the estimation of the M_rs of the analytes in our study. However, since we were not able to measure the intrinsic viscosity or radius of gyration by light scattering in our laboratory, all M_rs given for the water-soluble polymeric substances produced by *Phanerochaete chrysosporium* from free and wheat cell wall bound DCA using UV detection (see section 2.3) should be considered as "apparent", i.e. found by "estimation".

2.9. Statistical analysis

The statistical analysis (Student's *t*-test, coefficient of variation, precision, accuracy) of the HPSEC data was carried out using Sigma Plot software according to Hoque [12,13].

3. Results and discussion

3.1. Optimization of HPSEC

Prior to HPSEC optimization, the column system was several times equilibrated with water at a flow-rate of 0.5 ml/min. No separation of polymer standards (M_p 1500–95 600) was achieved on the selected column using 0.1 *M* Na_2HPO_4 (pH 11) as mobile phase. Lowering the pH from 11 to 9 did not result in any major improvement of the separation. However, water (pH 7.0) as a mobile phase showed some improvement of the separation of the polymer standards. Subsequently, the pH of aq. Na_2HPO_4 was adjusted to 7.0, and the effects of solute concentration on the size-exclusion behavior of the polymer standards were studied (Fig. 1). Fig. 1 shows the effects of $NaNO_3$ in the mobile phase [5 m*M* Na_2HPO_4 + 0.1% MeOH (pH 7.0)] on the size-exclusion behavior of the polystyrolsulphonate Na-salt standard with M_p 95 600. The signal intensity of the M_p peak at t_R 14.1 min increased from 21% at 10 m*M* $NaNO_3$ to 100% at 0 m*M* $NaNO_3$. The negative peak in front of the peak at t_R 21.5 min was completely eliminated using the mobile phase without $NaNO_3$ (Fig. 1). Therefore, the addition of $NaNO_3$ in the mobile phase was omitted.

Fig. 2 shows the separation of polystyrolsulphonate Na-salt narrow standards using the

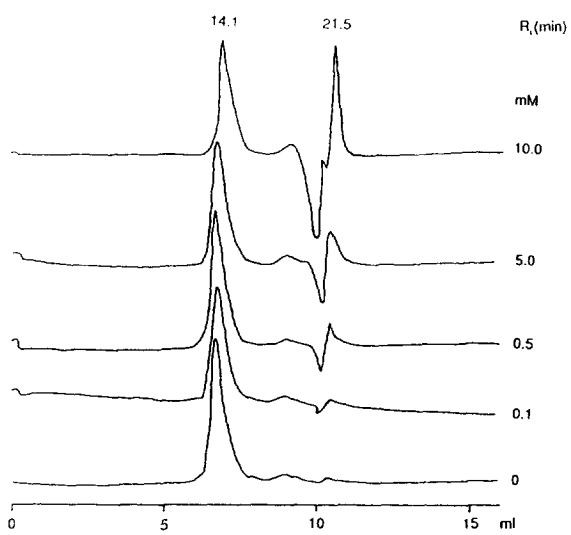

Fig. 1. Effects of $NaNO_3$ in the mobile phase [5 m*M* Na_2HPO_4 + 0.1% MeOH (pH 7.0)] on the size-exclusion behavior of polystyrolsulphonate Na-salt standard M_p = 95 600. Note the increment of the peak at t_R 14.1 min with elimination of the peak at t_R 21.5 min along with the negative peak in front of the peak at t_R 21.5 min.

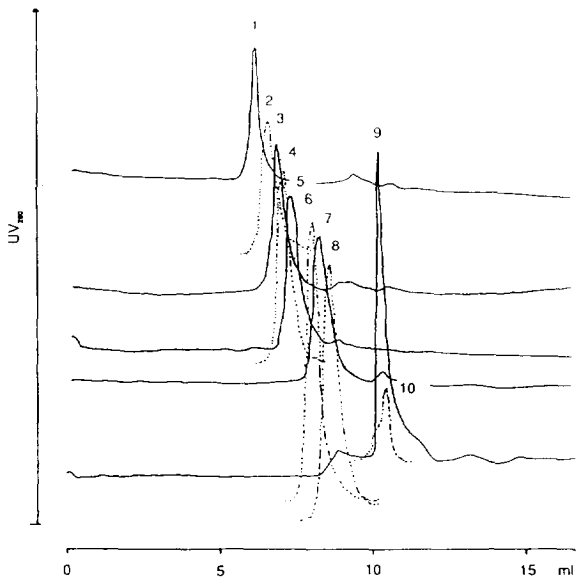

Fig. 2. Separation of polystyrol-sulphonate Na-salt narrow standards using the optimized mobile phase 5 m*M* Na_2HPO_4 + 0.1% MeOH (pH 7.0). The peaks of polystyrol-sulphonate Na-salt standards (indicated by solid and dashed lines) are designated by arabic numbers: 1 = M_p 400 000, 2 = M_p 200 000, 3 = M_p 95 600, 4 = M_p 73 500, 5 = M_p 46 000, 6 = M_p 19 300, 7 = M_p 8000, 8 = M_p 5400, 9 = M_p 1500, and 10 = M_p 102 (KNO_3). A sample equivalent to 50 μg standard was injected each time to obtain the chromatogram.

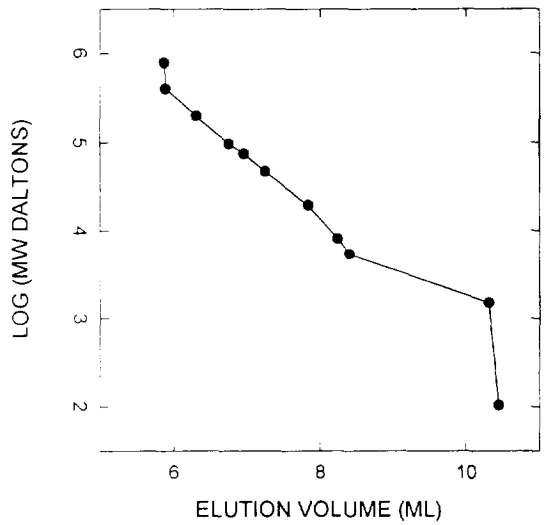

Fig. 3. Relationship between log M_p and elution volume (ml) using the optimized mobile phase 5 m*M* Na_2HPO_4 + 0.1% MeOH (pH 7.0).

optimized mobile phase 5 m*M* Na_2HPO_4 + 0.1% MeOH (pH 7.0). The signal-to-noise ratio was improved, the negative peak was eliminated and the difference in the t_Rs of the M_p 1500 and M_p 95 600 peaks was maximized.

The relationship between log M_p and retention volume V_R is shown in Fig. 3. The exclusion limit was found to be M_p 400 000 and the total permeation limit M_p 102.

After optimization of the HPSEC method for polystyrolsulphonate standards, the column was tested for the size-exclusion chromatography of Sitka spruce milled wood lignin, i.e. Spruce in situ lignin [7] (Fig. 4). The Sitka spruce milled wood lignin was shown to be polydisperse with M_p ranging from >102 to >400 000 (Fig. 4).

The standard deviation, precision and accuracy of V_R for polystyrolsulphonate Na-salt standards and Sitka spruce milled wood lignin components were found to be <0.2%, <2.9% and <7.5%, respectively. Thus, within the size-exclusion range between M_p 102 and M_p 400 000, the reproducibility of the size-exclusion chromatographic separation of synthetic (polystyrolsulphonate Na-salt) and natural (Sitka spruce milled wood lignin) polymer standards could be demonstrated.

3.2. Characterization of water-soluble polymeric substances produced by Phanerochaete chrysosporium from free [^{14}C]DCA and wheat cell wall bound [^{14}C]DCA

Fig. 5 shows the UV traces as well as the radioactivity traces of aq. soluble products derived from free [^{14}C]DCA, bound [^{14}C]DCA and free DCA + bound [^{14}C]DCA in the low-N cultures. The correspondence of the UV trace of lignin-like compounds (Fig. 5A) with the radioactivity trace (Fig. 5B) from free [^{14}C]DCA was either due to the polymerization of polar DCA-metabolites or due to the copolymerization of [^{14}C]DCA-degradation products with the ligninase-oxidized veratryl alcohol (veratrylaldehyde). For example, it is reported that ligninase may split the lignin between the C_α and the C_β of the propyl side chain to give benzaldehyde, which could be polymerized like veratrylaldehyde to soluble higher M_r products [14].

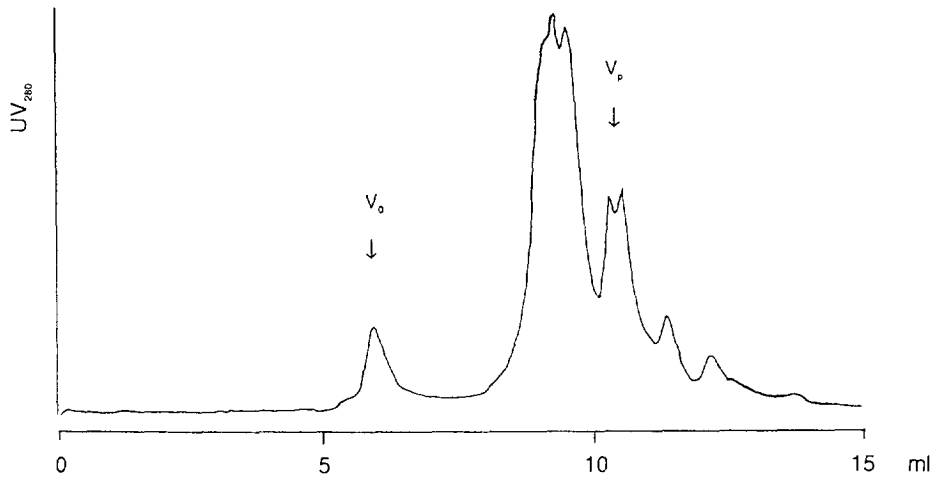

Fig. 4. Separation of sitka spruce milled wood lignin (109 μg in 0.1 *M* Na_2HPO_4, pH 11.18) using the optimized mobile phase 5 m*M* Na_2HPO_4 + 0.1% MeOH (pH 7.0).

The correspondence of the radioactivity trace (Fig. 5D) with the UV trace (Fig. 5C) of aq. soluble ^{14}C-products from bound [^{14}C]DCA showed their polydispersity to be approximately in the same range as that of free [^{14}C]DCA and free + bound [^{14}C]DCA in the low-N cultures of *Phanerochaete chrysosporium*. The low radioactivity trace (Fig. 5F) as compared to the UV trace (Fig. 5E) of the aq. soluble polymer from free DCA + bound [^{14}C]DCA was either due to a low concentration of bound [^{14}C]DCA applied in combination with free, non-labelled DCA (see Experimental) and/or due to increased depolymerization and mineralization of bound [^{14}C]DCA in the presence of free DCA.

In the low-C cultures characterized by lower mineralization rates [10], the radioactivity traces corresponded to the UV traces irrespective of the form (free or bound) and/or combination (free form + bound form) of applied [^{14}C]DCA (Fig. 6). However, similar to the low-N cultures (Fig. 5A,B), the correspondence of the radioactivity trace (Fig. 6B) with the UV trace (Fig. 6A) of the aq. soluble polymer from free [^{14}C]DCA indicated the occurrence of either polyphenolic-type polymerization of [^{14}C]DCA degradation products or copolymerization of [^{14}C]DCA degradation products with ligninase-produced veratrylaldehyde in low-C cultures.

The simultaneous change of the radioactivity trace and the UV trace at 280 nm of the aq. soluble polymer from bound [^{14}C]DCA of low-N and low-C cultures (Figs. 5 and 6) analogous to that from free [^{14}C]DCA suggested a substantial release of bound [^{14}C]DCA from wheat cell wall lignins via cleavage of labile bonds of [^{14}C]DCA to the C_α of lignin monomer side chains [11], and subsequent polyphenolic-type polymerization of [^{14}C]DCA degradation products by *Phanerochaete chrysosporium*.

3.3. Polydispersity of polymeric substances produced by Phanerochaete chrysosporium from [^{14}C]DCA and wheat cell wall [^{14}C]DCA–lignin metabolite fractions

Tennikov et al. [15] have shown that the polydispersity $U_w = (M_z)/(M_w)$ of unknown polymers could be determined by the formula:

$$\text{Polymer polydispersity, } U_w \approx 1 + \frac{\sigma_v}{\Psi(K)V_p} \qquad (1)$$

where σ_v is the width of the chromatogram, V_p is the total permeation volume, $\Psi(K)$ is the slope of the calibration curve, the mass-average molecular mass $M_w = \int_0^\infty f_w(M)\mathrm{d}M$ and $M_z = (M_w)^{-1}\int_0^\infty f_w(M)M^2\mathrm{d}M$ with $f_w(M)$ as the molecular mass distribution (MMD) function. According to Tennikov et al. [15], the slope of the calibration curve $\Psi(K)$ is dependent upon the

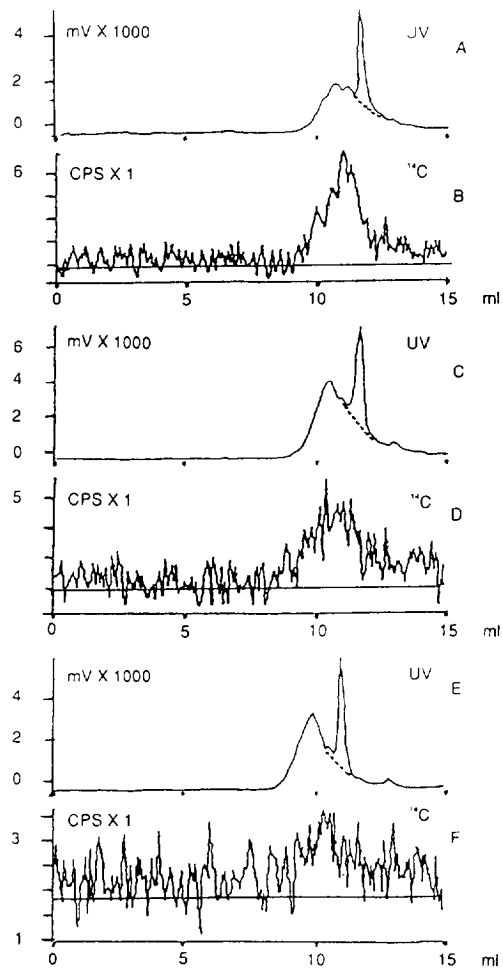

Fig. 5. Dual traces of aq. soluble lignin-like substances (UV at 280 nm) and radioactive substances from low-N cultures following size-exclusion chromatography using the optimized mobile phase 5 m*M* Na_2HPO_4 + 0.1% MeOH (pH 7.0): A = [^{14}C]DCA, UV 280 nm; B = [^{14}C]DCA, radioactivity trace; C = bound [^{14}C]DCA, UV 280 nm; D = bound [^{14}C]DCA, radioactivity trace; E = free DCA + bound [^{14}C]DCA, UV trace; F = free DCA + bound [^{14}C]DCA, radioactivity trace. For E and F, half of the concentrations of bound [^{14}C]DCA (25 μmole) instead of 50 μmole free or bound [^{14}C]DCA for A–D were applied (see Experimental) [10]. Due to the high intensity of the UV signals at 280 nm, the UV traces are given for 100-μl injection volumes for better UV signal resolution, whereas radioactivity traces obtained from 500-μl injection volumes are shown for improved signal-to-noise ratio. Dotted lines indicate the approximate correspondence of UV signal at low-intensity with the radioactivity trace, although at high intensity (500-μl injection volumes) the resolution of the UV signal was lost and corresponded fully to the radioactivity trace.

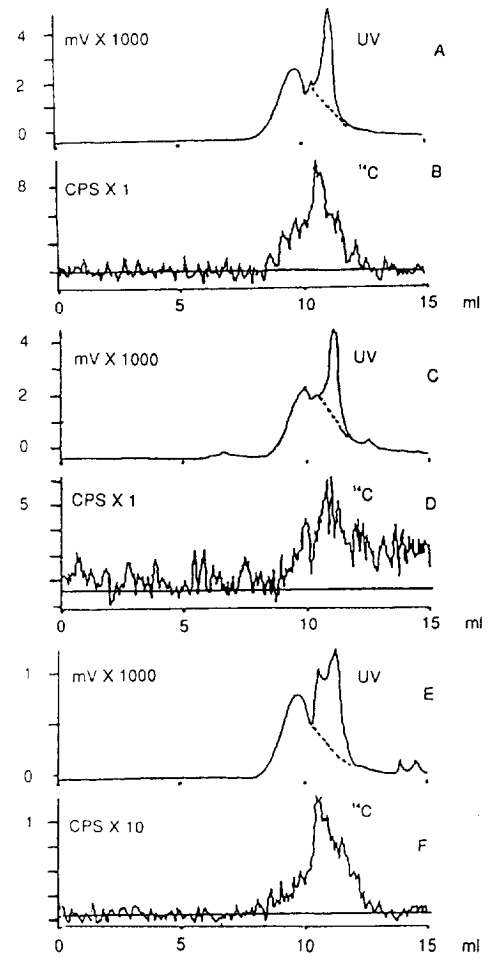

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Table 1
Determination of polydispersity (U_w) of polystyrolsulphonate Na-salt standards according to Tennikov et al. [15]

M_p	V_R (ml)	K	$\Psi(K)$	σ_v	U_w
95 000	6.76	0.08	0.19	2.20	2.22
73 500	6.96	0.10	0.21	2.20	1.98
46 000	7.25	0.13	0.24	1.70	1.46
19 300	7.84	0.19	0.27	2.50	1.76
8000	8.24	0.23	0.29	2.10	1.48
5400	8.41	0.24	0.29	2.20	1.52
1500	10.31	0.42	0.27	2.20	1.59

distribution coefficient $K = (V_R - V_0)/(V_p)$ with V_R = retention volume (ml), V_0 = void volume (ml) and V_p = total permeation volume (ml), and could be calculated by the equation:

$$\text{Slope of the calibration graph, } \Psi(K) \approx \begin{cases} \dfrac{1-K}{2}, & \text{if } K > 0.5 \\ K \ln\left(\dfrac{8}{\pi^2 K}\right), & \text{if } K < 0.5 \end{cases} \quad (2)$$

where $\pi \approx 3.1416$.

Using this calculation method, the polydispersity of polystyrolsulphonate Na-salt standards, Sitka spruce milled wood lignin as well as of aq. soluble polymeric substances produced by *Phanerochaete chrysosporium* from [^{14}C]DCA and wheat cell wall [^{14}C]DCA–lignin metabolite fractions was calculated. Table 1 shows that the polydispersity of narrow polystyrolsulphonate Na-salt standards lies within the range 1.48–2.22, which is typical for the narrow GPC standards [$(M_w)/(M_n) = 1.1–1.2$], where M_w is the mass-average molecular mass and M_n is the number-average molecular mass. Thus, the method described by Tennikov et al. [15] for the calculation of polydispersity of unknown polymers was found to be appropriate and justified for the calculation of polydispersity of unknown lignin-like substances in our study.

The polydispersity (U_w) and molecular masses (M_p) of aq. soluble lignin-like substances from the culture filtrates of *Phanerochaete chrysosporium* are summarized in Table 2. In both low-N and low-C cultures, the polydispersity and the molecular mass of aq. soluble lignin-like substances from cell wall bound [^{14}C]DCA were higher than those of free [^{14}C]DCA. The high molecular mass (M_p) of the aq. soluble lignin-like substances of bound [^{14}C]DCA from low-C cultures (Table 2) corresponded to their low mineralization rates [10]. In the low-N cultures, the polydispersity and the molecular mass of lignin-like substances from free DCA + cell wall bound [^{14}C]DCA were higher than those of cell

Table 2
Determination of molecular mass (M_p) and polydispersity (U_w) of lignin-like compunds from culture filtrates of *Phanerochaete chrysosporium* according to Tennikov et al. [15]

Sample	Approx. M_r range ($\times 10^3$)	Mean M_r	V_R^a (ml)	K	$\Psi(K)$	σ_v	U_w
Low-N cultures							
[^{14}C]DCA	0.1–3.7	2814	9.67	0.36	0.29	1.57	1.27
Bound [^{14}C]DCA	0.1–4.4	3081	9.54	0.35	0.29	1.85	1.36
Free DCA + bound [^{14}C]DCA	0.1–5.2	3471	9.35	0.33	0.30	2.22	1.51
Low-C cultures							
[^{14}C]DCA	0.1–15.9	3655	9.26	0.32	0.30	2.59	1.70
Bound [^{14}C]DCA	0.1–385.7	10 543	8.15	0.22	0.29	4.63	3.40
Free DCA + bound [^{14}C]DCA	0.1–15.9	3655	9.26	0.32	0.30	2.59	1.70

[a] Mean V_R of polydisperse signal of lignin-like substances within the size-exclusion limit.

wall bound [^{14}C]DCA, but in the low-C cultures no differences between the two groups could be found. One of the components of the so formed water-soluble polymer from free [^{14}C]DCA, bound [^{14}C]DCA and free DCA + bound [^{14}C]DCA was more polar than DCA–N-glucoside on the reversed-phase C_8 column (see section 2.4; data not shown).

4. Conclusions

The suitability of the described HPSEC method for the separation of lignin-like substances and the aq. soluble xenobiotic polymers is demonstrated. The described method is rapid, reproducible, and allows determination of molecular mass and the polydispersity of unknown water-soluble xenobiotic polymers. The polydispersity (U_w) and the mean molecular mass (M_p) of lignin-like substances were higher in low-C cultures with lower mineralization rates than those in low-N cultures with higher mineralization rates (Table 2) [10,16].

The release of [^{14}C]DCA aglucones from bound forms and the subsequent polymerization of [^{14}C]DCA degradation products (Figs. 5 and 6) did not depend primarily upon the efficiency of mineralization by *Phanerochaete chrysosporium* [10], e.g. in low-N and low-C cultures, but rather on its depolymerization efficiency of crosslinked wheat cell wall lignins, e.g. in low-N cultures as compared to low-C cultures, making labile bonds of [^{14}C]DCA with the C_α of cell wall lignin monomer side chains more accessible to enzymatic and non-enzymatic hydrolytic cleavage (Table 2). Thus, the release of [^{14}C]DCA via cleavage of labile bonds (benzylamine linkage) of DCA to the C_α of lignin monomer side chains [11,17] by *Phanerochaete chrysosporium* could predominately occur. If predominately stable bonds (benzylamine linkage) of DCA to the ring-C of lignin monomers on wheat cell walls [11] should have existed, the release of bound [^{14}C]DCA and the degree of polymerization of [^{14}C]DCA degradation products in low-N and low-C cultures by the white-rot fungus should be expected to be different (Figs. 5 and 6). Indeed, our results were supported by Lange [18] who found that labile bonds of DCA covalently bound to the C_α of the lignin monomer side chains in reference artificial lignin (DEHP)–DCA copolymers account for the entire (ca. 100%) DCA–lignin bonding pattern. Depolymerization of wheat cell wall bound DCA, metabolism and polymerization of released aglucones might be regarded as competitive processes of detoxification and/or mineralization [6].

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