Data Article

Dataset on structure-antioxidant activity relationship of active oxygen catalytic lignin and lignin-carbohydrate complex

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Abstract  
The data presented in this article are related to the research article entitled “Structure-antioxidant activity relationship of active oxygen catalytic lignin and lignin-carbohydrate complex” (Jiang et al.). It supplements the article with thermostability of milled wood lignin (MWL) and alkali-oxygen lignin (AOL), main substructures of lignin in rice straw, main products and yield of nitrobenzene oxidation of lignin-carbohydrate complexes (LCCs), Fourier transform infrared spectroscopy of LCCs, radical (ABTS-) scavenging ability of lignins and signal assignment of lignins and LCCs in nuclear magnetic resonance spectra (1H, 13C, 2D HSQC NMR). The dataset is made publicly available and can be useful for extending the structural and bioactive research and critical analyses of lignin and LCC.

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In this report, we present data on the structure-antioxidant activity relationship of lignin and LCC to supplement the analysis of our research article [1]. Thermostability is an important property of antioxidants to identify its antioxidant capacity, which was demonstrated by TGA as shown in Fig. 1. Spectroscopic methods (NMR and FTIR) combined with chemical degradation (nitrobenzene oxidation) can give comprehensive structural analysis of lignin and LCC. The signal assignment of NMR and FTIR spectra supplements the information of the main substructures (Fig. 2) of lignin in rice straw, which can be assigned and analyzed according to the published literatures [4–7]. Chemical degradation of nitrobenzene oxidation (Fig. 3) endows this research with monomeric composition and the condensation degree of lignin, and the raw data were listed in Table 4. The assessment of ABTS•- scavenging ability (Fig. 5) is used to prove the data of corresponding DPPH-assay and to demonstrate that the AOL has higher antioxidant activity.

2. Experimental design, materials, and methods

2.1. Thermostability and FTIR

The thermostability was determined by a thermogravimetric analyzer (SDT 650) using a heating rate of 5 °C/min in air from room temperature to 1000 °C.

FTIR spectra of LCCs were recorded using a FTIR spectrometer (VERTEX 80 V, Bruker, Germany). 1 mg of samples was mixed with 200 mg of KBr. After grinding and tableting, the FTIR spectra was recorded with the scan resolution of 4 cm⁻¹ and the scan area of 4000–400 cm⁻¹.
2.2. NMR characterization

MWL and AOL were acetylated according to the method reported by Lu and Ralph [8] for the determination of $^1$H and $^{13}$C NMR. 20 mg of acetylated lignins was dissolved in 0.5 mL DMSO-$d_6$ for $^1$H NMR detection. For the quantitative $^{13}$C NMR experiment, acetylated lignin (150 mg) was dissolved in DMSO-$d_6$ (0.5 mL). Chromium (III) acetylacetonate (20 μL, 0.01 M) was added to provide complete relaxation of all nuclei. The mixture was then transferred to a Shigemi microtube and characterized at 25 °C. The acquisition parameters were: 90° pulse width, a relaxation delay of 1.7 s, and an acquisition time of 1.2 s. A total of 20,000 scans were collected.

For 2D HSQC NMR test of LCCs, the LCC samples (50 mg) were dissolved in 0.5 mL of DMSO-$d_6$. The number of collected complex points was 2048 for the $^1$H-dimension with a recycle delay of 1.5 s. The number of transients was 64, and 256 time increments were recorded in the $^{13}$C-dimension. The $^1$J$_{CH}$ used was 145 Hz. Processing used typical matched Gaussian apodization in the $^1$H-dimension and squared cosine-bell apodization in the $^{13}$C-dimension. Prior to Fourier transformation, the data matrices were zero-filled to 1024 points in the $^{13}$C-dimension.

**Table 1**

| Label | $\delta_H$ (ppm) | Assignment |
|-------|-----------------|------------|
| 1     | 7.42–8.00       | Aromatic proton in p-hydroxyphenyl units |
| 2     | 6.75–7.42       | Aromatic proton in guaiacyl units |
| 3     | 6.15–6.75       | Aromatic proton in syringyl units |
| 4     | 5.69–6.15       | H$_a$ in β-O-4' and β-1' structure |
| 5     | 5.22–5.69       | H$_a$ in β-5' and α-O-4' structure |
| 6     | 4.48–5.22       | H$_a$ in β-β' structure |
| 7     | 4.01–4.48       | H$_a$ in β-O-4' structure |
| 8     | 3.43–4.01       | Proton in methoxyl |
| 9     | 2.15–2.42       | Proton in aromatic acetates |
| 10    | 1.58–2.15       | Proton in aliphatic acetates |
| 11    | 0.66–1.58       | Proton in –CH$_2$– and –CH$_3$ |
2.3. Nitrobenzene oxidation

Nitrobenzene oxidation was applied to the LCCs according to the procedure reported by Chen [2]. Briefly, 10 mg of sample was reacted with 0.25 mL nitrobenzene in a stainless steel bomb at 170 °C for 2 h under alkali condition (4 mL 2 mol/L sodium hydroxide). Then, the bomb was cooled in cold water immediately and 1 mL 0.1 mol/L sodium hydroxide solution containing 3-ethoxy-4-hydroxybenzaldehyde (0.3 g/L) was added as the internal standard. The mixture was extracted three times with dichloromethane in separating funnel. The aqueous phase was acidified with 4 mol/L HCl to pH = 1 and extracted twice with dichloromethane and once with ethyl ether. The combined organic

Table 3
Assignment of the polysaccharide signals in the 2D HSQC NMR spectra of LCCs.

| Label | $\delta_C$/$\delta_H$ (ppm) | Assignment |
|-------|-----------------|-------------|
| Est   | 66–62/4.5–4.0   | C–H in $\gamma$-ester linkages |
| Xs    | 62.9/3.41      | C$_2$–H$_2$ in $\beta$-D-xylpyranoside |
| X$_3$ | 72.7/3.05      | C$_2$–H$_2$ in $\beta$-D-xylpyranoside |
| X$_2$ | 73.1/4.50      | C$_2$–H$_2$ in 2-O-acetyl-$\beta$-D-xylpyranoside |
| X$_3$ | 73.7/3.29      | C$_2$–H$_2$ in $\beta$-D-xylpyranoside |
| X$_{31}$ | 74.9/4.81   | C$_3$–H$_2$ in 3-O-acetyl-$\beta$-D-xylpyranoside |
| X$_4$ | 75.5/3.53      | C$_4$–H$_4$ in $\beta$-D-xylpyranoside |
| BE$_4$ | 81.6/4.63    | C$_5$–H$_5$ in benzyl ether (secondary OH of carbohydrate) linkages |
| Ara$_4$ | 86.8/4.32   | C$_4$–H$_4$ in arabinan |
| $\alpha$X$_{31}$[(R)] | 92.5/4.89 | C$_1$–H$_1$ in (1→4)-$\alpha$-D-xylpyranoside (R) |
| $\beta$X$_{31}$[(R)] | 97.6/4.25 | C$_1$–H$_1$ in (1→4)-$\beta$-D-xylpyranoside (R) |
| X2$_3$ | 99.5/4.74    | C$_1$–H$_1$ in 2,3-O-acetyl-$\beta$-D-xylpyranoside |
| X$_2$ | 99.8/4.52     | C$_1$–H$_1$ in 2-O-acetyl-$\beta$-D-xylpyranoside |
| X$_3$ | 101.9/4.28    | C$_1$–H$_1$ in 3-O-acetyl-$\beta$-D-xylpyranoside |
| PhGlc$_1$ | 100.3/5.09 | C$_1$–H$_1$ in phenyl glycoside linkages |
| PhGlc$_3$ | 101.9/4.95 | C$_3$–H$_3$ in phenyl glycoside linkages |
| X$_4$/Glc$_1$ | 103.2/4.29 | C$_1$–H$_1$ in $\beta$-D-xylpyranoside/$\beta$-D-glucopyranoside |
The DPPH⋅ and ABTS⋅ radical scavenging assay of lignins and LCCs was performed using a spectrophotometric method. Samples were dissolved in 90% 1,4-dioxane/water (v/v). The DPPH⋅ was dissolved in anhydrous ethanol with the concentration of $6 \times 10^{-3}$ mol/L. ABTS⋅ was generated by reacting 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (7 mM) with 2.45 mM potassium persulfate (K$_2$S$_2$O$_8$) in ultrapure water and then letting the solution stand for 15 h in the dark at room temperature. The radical solution was adjusted to obtain an UV absorbance of 0.70 ± 0.02.
Fig. 3. The main products of alkali nitrobenzene oxidation of lignin.
at 517 nm and 734 nm for DPPH$^+$ and ABTS$^-$, respectively. The concentration of lignin and LCCs in tested sample is 0.03 mg/mL. The absorbance of tested sample was measured using a microplate spectrophotometer (Infinite M200, Ku nshan, China). The radical scavenging ability was calculated using the following formula:

\[
\text{Scavenging ability (\%) = \left[1 - \frac{(A_i - A_j)}{A_0}\right] \times 100}
\]

where \(A_i\) is the absorbance of the tested sample; \(A_j\) is the absorbance of the blank sample via anhydrous ethanol replacing DPPH$^+$ or ultrapure water replacing ABTS$^-$ solution; \(A_0\) is the absorbance of the blank sample via anhydrous ethanol or ultrapure water replacing lignin solution.

Table 4
The yield and ratio of nitrobenzene oxidation products of LCCs.

| Samples     | Yield (mmol/g-lignin) | V/S/H* |
|-------------|-----------------------|--------|
| LCC$_{RS}$  | 1.20 ± 0.01           | 0.41 ± 0.01 0.46 ± 0.00 2.07 ± 0.02 | 58/20/22 |
| LCC$_{AO}$  | 0.22 ± 0.00           | 0.18 ± 0.03 0.18 ± 0.01 0.58 ± 0.01 | 38/31/31 |

* V = vanillin + vanillic acid; S = syringaldehyde + syringic acid; H = p-hydroxybenzaldehyde + p-hydroxybenzoic acid.

Table 5
The position and assignment of absorption peaks in LCCs.

| Wavenumber (cm$^{-1}$) | Assignment                              |
|------------------------|----------------------------------------|
| 1724                   | Stretching vibration of non-conjugate C=O |
| 1641                   | Stretching vibration of conjugate C=O   |
| 1505                   | Stretching vibration of benzene ring    |
| 1462                   | Bending vibration of C–H (CH$_2$, CH$_3$) |
| 1401                   | Stretching vibration of benzene ring    |
| 1263                   | Stretching vibration of C–O in G-unit   |
| 1160                   | Stretching vibration of phenolic acid ester |
| 1086                   | Bending vibration of C–H and C–O       |
| 840                    | Out-of plane bending vibration of C–H in benzene ring (S/H) |

Fig. 4. FTIR spectra of LCCs.
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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Fig. 5. The ABTS scavenging ability of MWL and AOL.