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Polyfunctional Nanometric Particle Obtained from Lignin, Woody Biomass Resource

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Experimental

Sample preparation

_Simultaneous enzymatic saccharification and physical comminution (SESPC):_ The detailed procedure was presented in a footnote in the main text and the patents are shown in ref. S1. The SESP was performed upon Japanese cedar using a stemmill® (LME 3000; Ashizawa Finetech Ltd., Japan).

_Purification of SESP-lignin:_ The black liquor obtained by the SESP process was purified by ultrafiltration and rinsed with deionized water on a 100 nm Millipore filter. The residue was then collected as a black powder.

_Acetylation of SESP-lignin:_ The 2.01 g of lignin was mixed with 5.6 ml of acetic anhydride in 140 mL of dimethyl acetamide (DMAc). The mixture was dissolved by 30 min of ultrasonication and was then heated at 120 °C for 18 h. The products were purified by reprecipitation from toluene. The products were collected via vacuum filtration on a 100 nm Millipore filter and dried in vacuo at 100 °C for 6 h. The acetylated lignin was obtained as a blown colored powder (52%). [Note: The yield of acetylation is stoichiometiric except for mechanical (filtration) losses. One can increase the yield by evaporating the reaction mixture in prior to reprecipitation.]

Characterizations

_Solubility test:_ The 50 mg of SESP-lignin was put into 3.5 mL of solvent (equal to 1.4 wt.-% SESP-lignin solution). The sample was sonicated at r.t. for 30 min and was then allowed to stand for about 1 h.

_Transmission electron microscopy (TEM)._ TEM observation was performed by using a JEM-2100 (JEOL, Tokyo, Japan) at an acceleration voltage of 200 kV. 5 μl of SESP-lignin solution was dropped on carbon-coated grids (Nisshin EM Co., Tokyo). After 1 min on the grids, 2% (w/v) uranyl acetate was added to the sample momentarily, and the grids were then air-dried and employed for observation.
Dynamic light scattering (DLS): DLS measurement was performed using a Zetasizer Nano ZS equipped with a 4 mW He-Ne laser ($\lambda = 632$ nm) (Malvern Instruments Ltd., United Kingdom). The SESPC-lignin solution used was measured with a solubility test.

Gel permeation chromatography (GPC): GPC measurements were performed on a JASCO system (HPLC LC-2000 Plus series) equipped with polystyrene gel columns (SKgel G3000HHR, TOSOH, Japan) using N,N-dimethyl formamide (DMF) as an eluent after calibration with poly(styrene) standard ($M_w = 4000, 50000, 200000$; Polyscience, Inc., U.S.A). The sample and poly(styrene) standard was dissolved in DMF.

Fourier transform infrared (FT-IR) spectra: FT-IR spectra were recorded on a JASCO FT/IR-4100 spectrometer for KBr pellets.

Nuclear magnetic resonance spectroscopy (NMR): $^1$H-NMR spectra were measured on a JEOL AL500 MHz spectrometer at 20 ºC. Chemical shifts are reported in ppm downfield from SiMe$_4$, using the solvent’s residual signal as an internal reference.

Thermogravimetric analysis (TGA): TGA was carried out on a Rigaku Thermo plus TG8129, under nitrogen flow, at a scanning rate of 10 ºC min$^{-1}$. 
**Fig. S1** GPC profiles of the mixture of (a) SESPC-lignin and (b) acetylated SESPC-lignin with poly(styrene) (PS)-standards. (1) SESPC-lignin or acetylated SESPC-lignin, (2) PS of $M_W = 4000$, (3) PS of $M_W = 50000$ ($M_W/M_n = 1.06$), and (4) PS of $M_W = 200000$ ($M_W/M_n = 1.06$). The result of GPC indicates that the weight-average molecular weight ($M_w$) of SESPC-lignin is below 4,000, which is smaller than that reported in other lignocellulose.\(^4\) Since one sharp peak was confirmed in the GPC chart of SESPC-lignin, it is considered that the SESPC-lignin is monodispersed, as shown in the DLS results (Fig. 3). The long elution time of SESPC-lignin on GPC would be due to its particle shape. The peak top of acetylated SESPC-lignin moves shorter elution time and the peak becomes broader than that of native SESPC-lignin. This would be because of an increase in particle size of lignin (shown in Fig. 4) which is induced by the change in the swelling property of the SESPC-lignin particles, i.e., by increasing of the solvation and gel-like character of the particles.
**Fig. S2** FT-IR spectra of (a) SESPC-lignin, and (b) acetylated SESPC-lignin. The emergence of a peak at 1740 cm\(^{-1}\) (the colored peak) corresponds to the acetylation of the phenolic and aliphatic hydroxyl groups in SESPC-lignin.
Fig. S3 $^1$H-NMR spectra of SESPC-lignin (a) before, and (b) after acetylation. It is considered that the doublet peak at 1.94 and 1.90 ppm (indicated by red arrow) originated from the acetyl-CH$_3$ groups, indicating that these peaks also support the progress of acetylation.
**Fig. S4** Solubility of acetylated SESPC-lignin for chloroform in which 50 mg of SESPC-lignin before (left) and after (right) acetylation in 3.5 mL of solvent (equal to 1.4 wt.-% SESPC-lignin solution).
Fig. S5 TG-DTA result for SESPC-lignin. The decomposition temperature of SESPC-lignin is 250 ºC. Furthermore, the residue after heating at 500 ºC is 60 wt.-%. By heating until 1000 ºC, the residue vanishes completely.
References