Polyfunctional nanometric particles obtained from lignin, a woody biomass resource†

Kazuhiro Shikinaka,[a] Nozomu Fujii,[a] Shun Egashira,[a] Yoshihiko Murakami,[a] Masaya Nakamura,[b] Yuichiro Otsuka,[b] Seiji Ohara[b] and Kiyotaka Shigehara*[a]

Received 24th May 2010, Accepted 26th August 2010
DOI: 10.1039/c0gc00140f

Highly monodispersed nanometric particles, with a polyfunctional nature due to the presence of phenolic and aliphatic hydroxyl groups, were obtained by the simultaneous enzymatic saccharification and physical comminution of lignin, which is a woody biomass resource.

The utilization of waste biomass is important for energy and material innovation. For example, it offers the possibility of creating useful material from waste biomass, which would enable us to replace artificial, non-renewable resources with natural, renewable resources.[1–3] Lignin is a typical waste biomass that has a three-dimensional network structure of the aromatic group, and a number of phenolic and aliphatic hydroxyl groups.[2a] Great quantities of lignin are extracted from a variety of plants. Its application, however, has been limited to a few cases[1d] because of difficulties in its transformation to a low molecular weight molecule and the removal of impurities (*e.g.*, cellulose and hemicellulose) without causing denaturation of the functional groups.[2f,4] Intact low molecular lignin that is soluble in chloroform has recently been obtained by the simultaneous acetylation and ball milling of plant cell walls.[5] This procedure, however, still only yields lignocelluloses, *i.e.*, a mixture of lignin and cellulose, the existence of which usually causes a lack of heat resistant properties upon materialization.

Recently, we performed a simultaneous enzymatic saccharification and physical comminution (hereafter denoted as SESPC) for the efficient fermentation of sugar.[6]‡ Surprisingly, the ratio of saccharification of SESPC exceeds 85%, and the fermentation of the saccharified solution gives ethanol with a yield of approx. 100% relative to the theoretical value. Prior to the saccharification process, the lignin was extracted as a black powder.

Since the saccharification was performed enzymatically with an extremely high ratio, it was expected that the lignin obtained (hereafter referred to as SESPC-lignin) would be pure and lowly-degradative in nature. Until now, the main source of lignin (Kraft lignin) has been as a byproduct from the conversion of wood chips to cellulosic fibers through the Kraft process.[7] In contrast, SESPC-lignin is obtained by a process of saccharification of cellulose and hemicelluloses. In particular, it is expected that SESPC-lignin would be pure relative to Kraft lignin because the saccharification by SESPC takes place with a high ratio. Confirmation of the properties of SESPC-lignin is therefore important in order to demonstrate the feasibility of its usage as a material and to better understand the nature of lignin. In this Communication, the properties of SESPC-lignin are studied in detail.

As shown in Fig. 1, SESPC-lignin is readily soluble in polar organic solvents such as *N*,*N*-dimethyl formamide (DMF), dimethyl acetamide (DMAc) and dimethylsulfoxide (DMSO). SESPC-lignin is partly soluble in water, methanol (MeOH), acetone and tetrahydrofuran (THF). In particular, SESPC-lignin clearly separates into soluble and insoluble parts in water and MeOH, but in contrast, it never dissolves in non-polar organic solvents such as benzene, toluene, hexane and chloroform. This solubility of SESPC-lignin in only polar organic solvents suggests that little denaturation occurs on the phenolic groups during the SESPC process, and that there are few impurities, such as (hemi)cellulose, in SESPC-lignin.

Fig. 1 Solubility of SESPC-lignin in various solvents, in which 50 mg of SESPC-lignin was placed into 3.5 mL of solvent (equal to a 1.4 wt% SESPC-lignin solution).

Observations by transmission electron microscopy (TEM) clearly indicate that ESPSC-lignin is a particle (Fig. 2).

In the TEM image, the particle size is smaller than 100 nm. Surprisingly, dynamic light scattering (DLS) measurements revealed that the polydispersity index (PI) of SESPC-lignin in DMAc is 0.155 (Fig. 3(a)), *i.e.*, the SESPC-lignin is a monodispersed particle. The monodispersity of SESPC-lignin was also confirmed by gel permeation chromatography (GPC) measurements, as shown in Fig. S1(a).† Furthermore, the peak for SESPC-lignin is at approx. 260 nm, which is considerably smaller than that of lignin or lignocellulose (10 μm ~), as was reported previously. The peak changes with the duration of the SESPC process, from 1 mm to 260 nm (data not shown). These results encouraged us to use SESPC-lignin as a

---

[a] *Graduate School of Engineering, Tokyo University of Agriculture and Technology, Koganei, Japan. E-mail: jun@cc.tuat.ac.jp; Fax: +81 42-381-8175; Tel: +81 42-388-7052*
[b] *Forestry and Forest Products Research Institute, Tsukuba, Japan. E-mail: nmasaya@ffpri.affrc.go.jp; Fax: +81 029-873-3797; Tel: +81 29-829-8282*

† Electronic Supplementary Information (ESI) available: Experimental section, GPC charts, TG-DTA results, FT-IR spectra, NMR spectra and solubility tests of acetylated SESPC-lignin. See DOI: 10.1039/c0gc00140f

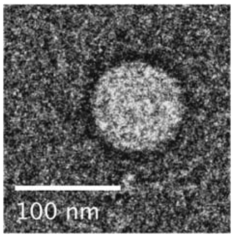

Fig. 2 A TEM image of SESPC-lignin. Since the specimen was prepared by a negative staining method, the brighter region can be considered to be SESPC-lignin.

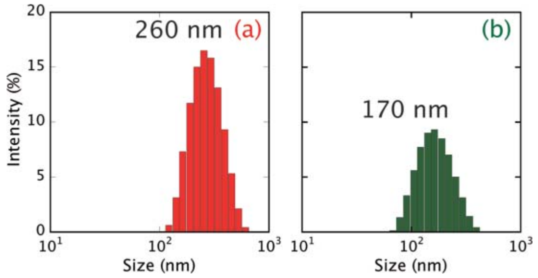

Fig. 3 Dynamic light scattering (DLS) results for (a) a 1.4 wt% SESPC-lignin DMAc solution and (b) the soluble part of SESPC-lignin in MeOH.

uniformly-sized nanometric material. The soluble part of SESPC-lignin in MeOH has a peak at a smaller size (approx. 170 nm; Fig. 3(b)). Thus, the results of DLS indicate that the size of an SESPC-lignin particle changes depending on the solvent, *i.e.*, SESPC-lignin's affinity for a given solvent has an effect upon its size.

The particle size of SESPC-lignin, as measured by DLS, is about twice as large as that measured by TEM. Since the TEM samples were prepared by negative staining through the drying process (see the ESI†), this result suggests that the particle size is diminished during the drying of the solvent, *i.e.*, the SESPC-lignin is a gel particle that has a three-dimensional network structure of aromatic groups.

Because lignin has both phenolic and aliphatic hydroxyl groups, its chemical modification has been widely studied by previous researchers.[8] The acetylation of SESPC-lignin is able to undergo heating with acetic anhydride in DMAc at 120 °C for 18 h. The acetylation of SESPC-lignin was confirmed by FT-IR (Fig. S2†) and ^{1}H NMR (Fig. S3†) spectra. These results suggest that SESPC-lignin is a nanometric particle with a polyfunctional character, which encouraged us to employ it as a macromolecular additive to polymer materials. Since the solubility of SESPC-lignin in chloroform drastically increases upon acetylation (Fig. S4†), the acetylation of SESPC-lignin allowed us to apply lignin particles to nanocomposite materials with various organic polymers. The particle size of the acetylated SESPC-lignin was approx. 460 nm, which is larger than that of non-modified SESPC-lignin (Fig. 4). The increase in particle size is due to changes in the swelling properties of the SESPC-lignin particles, as acetylation may increase the solvation and give a gel-like character to the particles.

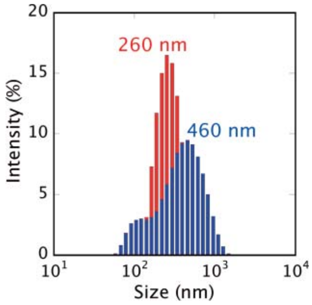

Fig. 4 DLS results for a 1.4 wt% acetylated SESPC-lignin chloroform solution (blue histogram) and a 1.4 wt% SESPC-lignin DMAc solution (red histogram; the same data as in Fig. 2(a)). The PI of acetylated SESPC-lignin in chloroform is 0.271. This result indicates that the size distribution of SESPC-lignin particles is broadened due to changes in its swelling properties and the low molecular weight by-product by acetylation, which corresponds to the GPC results (Fig. S1(b)†).

Conclusions

In conclusion, nanometric lignin particles were obtained by the simultaneous enzymatic saccharification and physical comminution (SESPC) method. The lignin particles obtained by SESPC (SESPC-lignin) are soluble in polar organic solvents due to their high purity and low-degradative nature. Furthermore, the size of the SESPC-lignin particles are highly mono-dispersed. Since SESPC-lignin also has a polyfunctional nature due to its phenolic and aliphatic hydroxyl groups, it offers the prospect of applications in various nanosciences and -technologies, *e.g.*, drug delivery systems (such as core–shell type nanometric micelles) and macromolecular additives. Because SESPC-lignin displays flame retardant properties (Fig. S5†), we can expect it to be used as a flame retardant material and as a nanocomposite material for organic polymers.

Although the utilization of lignin has been investigated by many researchers,[8,9] there have been a number of difficulties upon utilization due to its denaturation prior to materialization or due to unremovable (hemi)cellulosic components that cause a lack of heat-resistant properties. As shown above, the lignin presented here is in the form of polyfunctionalized nanometric particles with a uniform size. Furthermore, the SESPC method can, in principle, be performed on an industrial scale. Thus, the lignin obtained by this SESPC method encourages us to pursue innovation with this material to serve both industry and technology.

This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Rural Biomass Research Project, BM-D1310). We thank Mr Yusuke Uchida (Tokyo University of Agriculture and Technology) for assisting us with the GPC measurements.

Notes and references

‡ Simultaneous enzymatic saccharification and physical comminution (SESPC) was performed as follows. The physical comminution of wood powder after alkali cooking with anthraquinone was undertaken by bead milling with cellulase, hemicellulase and a buffer (pH = 5.0) for enzymatic saccharification at 50 °C for ~3 d. The saccharified solution and lignin were then obtained as a supernatant and a black liquor, respectively.

1 (*a*) A. J. Ragauskas, C. K. Williams, B. H. Davison, G. Britovsek, J. Cairney, C. A. Eckert, W. J. Frederick, Jr., J. P. Hallett, D. J. Leak, C.

L. Liotta, J. R. Mielenz, R. Murphy, R. Templer and T. Tschaplinski, *Science*, 2006, **311**, 484; (*b*) E. T. H. Vink, K. R. Rabago, D. A. Glassner and P. R. Gruber, *Polym. Degrad. Stab.*, 2003, **80**, 403; (*c*) Y. Nishio, *Adv. Polym. Sci.*, 2006, **205**, 97; (*d*) S.-i. Hirota, T. Sato, Y. Tominaga, S. Asai and M. Sumita, M., *Polymer*, 2006, **47**, 3954.

2 (*a*) *Biopolymers*, ed. A. Steinbuchel, Y. Doi and M. Hofrichter, Wiley-VCH, Weinheim, Germany, vol. 3, 2002; (*b*) D. Riggile, *BioCycle*, 1998, **39**(3), 64; (*c*) B. Kamm, *Angew. Chem., Int. Ed.*, 2007, **46**, 5056; (*d*) J. N. Chheda, G. W. Huber and J. A. Dumesic, *Angew. Chem., Int. Ed.*, 2007, **46**, 7164; (*e*) T. Kaneko, T. H. Thi, D. J. Shi and M. Akashi, *Nat. Mater.*, 2006, **5**, 966; (*f*) M. Funaoka, *Polym. Int.*, 1998, **47**, 277; (*g*) A. Corma, S. Iborra and A. Velty, *Chem. Rev.*, 2007, **107**, 2411; (*h*) S. R. Boethling, E. Sommer and D. DiFiore, *Chem. Rev.*, 2007, **107**, 2207.

3 (*a*) *Biorefineries—Industrial Processes and Products*, ed. B. Kamm, P. R. Gruber and M. Kamm, Wiley-VCH, Weinheim, Germany, 2006; (*b*) Y. Román-Leshkov, C. J. Barrett, Z. Y. Liu and J. A. Dumesic, *Nature*, 2007, **447**, 982.

4 H. L. Hergert, G. C. Goyal, J. H. Lora, in *Lignin: Historical Biological and Materials Perspectives*, ed. W. G. Glasser, R. A. Northey and T. P. Schultz, American Chemical Society, Washington, USA 2000, pp. 265.

5 F. Lu and J. Ralph, *Plant J.*, 2003, **35**, 535.

6 (*a*) M. Nojiri, T. Ikeda, T. Sugimoto and K. Magara, *JP Pat.* 2008-092910; (*b*) T. Yamada and A. Kudo, *JP Pat.* 2008-308530.

7 J. Gierer, *Wood Sci. Technol.*, 1980, **14**, 241–266.

8 (*a*) J. J. Meister, *J. Macromol. Sci., Part C*, 2002, **42**(2), 235; (*b*) H. Dtruszczyk, in *Lignin: Properties and Materials*, ed. W. G. Glasser, American Chemical Society, Washington, USA 1989, pp. 245, 324 and 506; (*c*) W. G. Glasser, in *Adhesives from Renewable Resources*, ed. R. W. Hemingway, A. H. Conner and S. J. Branham, American Chemical Society, Washington, USA 1989, pp. 43.

9 R. A. Northey, in *Materials and Chemicals from Biomass*, ed. D. S. Argyropoulos, American Chemical Society, Washington, USA 1992, pp. 146.