Relationship between the Physical Properties and Surface Area of Cellulose Derived from Adsorbates of Various Molecular Sizes.

Hiroshi Ougiya,† Nobuya Hioki,†† Kunihiko Watanabe,††† Yasushi Morinaga,††† Fumihiro Yoshinaga, and Masahiro Samejima*  

Bio-Polymer Research Co. Ltd., KSP R&D Business Park Bldg. B-1015, 3-2-1 Sakato, Takatsu-ku, Kawasaki-shi, Kanagawa 213-0012, Japan  
*Department of Biomaterials Sciences, School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan  

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An aqueous suspension of bacterial cellulose (BC) has such physical properties as higher viscosity, emulsion-stabilizing effect and filler retention than cellulose of other origins.  

The specific surface areas of BC, microfibrillated cellulose and wood pulp were evaluated by determining the maximum amounts of adsorption of Congo red, cellulose dehydrogenase (CDH) and xylglucan. There was a positive linear correlation between the above-mentioned physical properties of each cellulose sample and the specific surface area derived from the maximum amount of CDH adsorbed. The highest physical property values for BC result from the largest external surface area of the fibrils of BC to which CDH was adsorbed.  

Key words: bacterial cellulose; cellulose fiber; suspension properties; specific surface area; maximum amount of adsorption  

Some strains of Acetobacter produce bacterial cellulose (BC) as an extracellular polysaccharide. Under static culture conditions, BC (St-BC) is produced as a gelatinous membrane at the surface of the culture medium, whereas under agitated conditions, BC (Ag-BC) is produced as particles that are well dispersed in the culture medium and form a suspension. Both Ag-BC and St-BC are composed of ribbon-shaped fibrils, less than 100 nm wide.† Suspensions of Ag-BC in particular, as well as of St-BC produced by disintegration with a homogenizer, show some physical properties that are desirable for industrial applications such as higher viscosities, emulsion-stabilizing effects and filler retention than those of microfibrillated cellulose (MFC).†† The physical properties of both BCs just described have been suggested to depend on the specific surface area of a fibril accessible to other fibrils, oil droplets or fillers used for papermaking.‡,‡‡  

Little is known about the relationships between the specific accessible surface areas and physical properties of cellulose fibrils. Solute exclusion and nitrogen adsorption methods have been used to measure the specific surface area of wood pulp,§,¶ and the specific surface area has been reported to be related to the dyeability and susceptibility to enzymatic degradation of wood pulp and cotton.§,¶  

However, Gama et al. have pointed out that the solute exclusion method evaluated only the surface area corresponding to the pores, and proposed that the nonporous surface, which they called 'external surface', was related to the susceptibility of cellulose fibrils to hydrolysis by cellulase.††§ We tried evaluating the surface area of some cellulose samples from the maximum amount of adsorption of molecules whose molecular sizes were quite different from one another.  

The aim of this study is to clarify the relationship between the surface area and physical properties (viscosity, emulsion-stabilizing effect and filler retention) of various cellulose samples and to explain why the physical properties of disintegrated Ag-BC suspensions are superior to those of other cellulose samples. 

Materials and Methods  

Materials. In order to compare the cellulose samples themselves in pure form without other ingredients, the following cellulose origins were used as adsorbents: St-BC, Ag-BC, MFC (Celish FD-100F; Daisel Chemical Industries, Tokyo, Japan) and LBKP (bleached hardwood kraft pulp; Celulose Nipo-Brasileira, Brazil). Scanning electron micrographs of the cellulose samples are shown in Fig. 1.  

St-BC and Ag-BC were produced by Acetobacter xylignum subsp. sucrofermentans BPR2001 cultured either statically in a Roux flask or in a jar fermenter under agitation as described elsewhere.††§ After the cultivation, both St-BC and Ag-BC were purified by soaking in a 0.1 N NaOH solution at 80°C for 20 min.††§  

St-BC, Ag-BC, MFC and LBKP (0.5% w/v) were disintegrated with a homogenizer (Oster Blender; Sunbeam-oster Household Products Co., USA) at 16,800 rpm for 1 min.  

The adsorbates used were a dye (Congo red; Nacalai Tesque, Kyoto, Japan), cellulose dehydrogenase (CDH), which was derived from Phanerochaete

† To whom correspondence should be addressed: Hiroshi Ougiya; FAX: +81-298-64-4310; E-mail: QYC03122@nifty.ne.jp.  
‡ Present address: Mitsubishi Paper Mills Ltd., 46 Wada, Tsukuba, Ibaraki 300-4247, Japan  
†† Present address: Mitsubishi Paper Mills Ltd., 1-4-1 Higashikamamachi, Katsushika-ku, Tokyo 125-0041, Japan  
††† Present address: Ajinomoto Co. Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki 210-8681, Japan
chrysosporium and prepared as described by Samejima et al., and xyllo glucan (Megazyme, Sydney, Australia). The molecular weights of Congo red and CDH were 696 and 89,000, respectively, and the average molecular weight of xyllo glucan was 980,000. These three materials are known to be specifically adsorbed to the surface of the cellulose molecule according to Langmuir’s adsorption theory.

Analysis of the physical properties of cellulose suspensions. Viscosity: The absolute value of the complex viscosity was measured with dynamic liquid viscoelasticity measuring apparatus (Fluids Spectrometer RFS II; Rheometric Scientific Ltd.). An aqueous suspension containing a 1.0% (w/v) cellulose sample was sandwiched between 5-cm-diameter parallel discs, which were oscillated at ten angular frequency increments over the range of 1 to 100 rad/s, at 30°C and at a strain of 10% in the frequency sweep mode, and then the viscosity was measured at an angular frequency of 10 rad/s.

Filler retention: Filler retention means the ratio of the amount of filler retained in paper to the total amount of filler supplied during the papermaking process, this being evaluated by the method of Hioki et al. Emulsion stability index (ESI): The leveling-off ratio of the amount of the emulsified phase to the total amount of the mixture is defined as ESI, which was measured by the method of Ougiya et al.

Specific surface area. A 0.033% (w/v) cellulose sample in a 100 mM phosphate buffer (pH 6.0) was incubated with various concentrations of Congo red at 30°C, after which the cellulose fibrils were removed by centrifugation at 15,000 rpm for 5 min, and the amount of Congo red in the supernatant was evaluated from the absorbance at 492 nm. The maximum amount of Congo red adsorbed was calculated by using equation 1 derived from Langmuir’s adsorption theory:

\[
[E]/[A] = 1/(K_{ad}[A]_{max}) + (1/[A]_{max})[E] 
\]

where \([E](\text{mg/mL})\) is the concentration of Congo red at adsorption equilibrium, \([A](\text{mg/g of cellulose sample})\) is the amount of Congo red adsorbed to the cellulose surface, \([A]_{max}\) (mg/g of cellulose sample) is the maximum amount of adsorption of Congo red to the cellulose surface, and \(K_{ad}\) is the adsorption equilibrium constant.

The amount of CDH adsorbed was determined by using the method of Samejima et al., and \([A]_{max}\) was calculated by using equation 1.

The amount of xyllo glucan adsorbed was determined by using the iodine/sodium sulfate method of Kooiman, and \([A]_{max}\) was calculated by using equation 1.

The surface area of a cellulose sample occupied by one molecule of each adsorbate was calculated in order to evaluate the specific surface area of each cellulose sample. The area occupied by a Congo red molecule was calculated on the basis of the adsorption model of Woodcock et al. under the assumption that each Congo red molecule adsorbs parallel to the cellulose molecule.

The area occupied by a CDH molecule was derived from the spheroidal molecular structural model proposed by Lehner et al. under the assumption that each CDH molecule would be adsorbed to the cellulose surface at one of its ends perpendicular to its longitudinal axis. The area occupied by a xyllo glucan molecule was calculated on the basis of the adsorption model proposed by Hayashi et al. under the assumption that each xyllo glucan molecule adsorbs parallel to its longitudinal axis and covers three adjacent cellulose molecules. The areas occupied by one molecule of Congo red, CDH and xyllo glucan were calculated to be 1.73, 19.6 and 1,870 nm², respectively.

The specific surface area (Ss) is expressed as follows:

\[
S_{s} = \frac{[A]_{max} \cdot N \cdot S_{a}}{Mw \times 10^{21}} 
\]

where \(M_{w}\) is the molecular weight of the adsorbate, \(N\) is Avogadro’s constant and \(S_{a}\) is the area occupied by one adsorbate molecule.

The radius of gyration of xyllo glucan molecules was measured by gel permeation chromatography (GPC) in order to estimate the size of the xyllo glucan molecules in a solution. The intrinsic viscosity at various elution times was measured by a GPC system equipped with a viscosity detector, and the absolute molecular weights at the same elution times were determined by GPC and a low-angle laser light scattering method. The relationship between the radius of gyration and the absolute molecular weight was determined by using Flory’s equation:

\[
[n] = \phi \left( \frac{6R_{g}^{2}}{M} \right)^{3/2} \cdot M^{1/2} 
\]

where \([n]\) is the intrinsic viscosity, \(\phi\) is Flory’s constant (2.86 \times 10^{-5}), \(R_{g}\) is the radius of gyration and \(M\) is the absolute molecular weight.
The radius of gyration of the xyloglucan we examined was approximately 40 nm.

Results

Table 1 shows absolute values of the complex viscosity (hereafter called viscosity), filler retention and ESI of each cellulose suspension tested. The values for the filler retention of BC and MFC were higher than that of LBKP. This result means that BC and MFC both improved filler retention when added to the stock suspension of pulp and other ingredients that is used for papermaking.\textsuperscript{4,22} Of the cellulose samples we examined, Ag-BC had the highest values of the above-mentioned physical properties, followed by St-BC (Table 1).

Table 2 shows the maximum amounts ([A]_{max}) of Congo red, CDH and xyloglucan adsorbed to the cellulose surfaces, and the specific surface areas of the cellulose samples. As the adsorption of Congo red, CDH and xyloglucan followed Langmuir’s adsorption theory, these adsorbates seem to have been adsorbed to the surface of each cellulose sample as monolayers. Therefore, the specific surface area could be evaluated from the product of the maximum amount of adsorption and surface area of cellulose fibrils occupied by the corresponding adsorbate molecule. Calculation of the specific surface areas derived from the Congo red, CDH and xyloglucan adsorption values ranged from 91 to 160, 1.5 to 30 and 26 to 311 m\(^2\)/g, respectively. The specific surface areas of BCs derived from Congo red were almost the same as that of MFC or LBKP, but those of BCs derived from the CDH and xyloglucan adsorptions were 5- to 10-fold larger than that of MFC of LBKP.

Figure 2 shows relationships between the area of cellulose fibrils occupied by one molecule of each adsorbate (horizontal axis) and the specific surface area of each cellulose sample shown in Table 2 (vertical axis). As the area of cellulose fibrils occupied by one molecule of each adsorbate reflects the size of each adsorbate molecule, the specific surface area of each cellulose sample varied according to the size of the adsorbate molecule. The specific surface area derived from CDH adsorption (plots at 19.6 on the horizontal axis) was smaller than that derived from Congo red adsorption (plots at 1.73 on the horizontal axis).

Discussion

Figure 3 shows diagrams of the surface structures of the four cellulose samples constructed on the basis of the following knowledge: MFC is produced from plant cellulose fibers by mechanical fibrillation\textsuperscript{23} and consists of fibriller bundles ranging from 100 nm to 20 \(\mu\)m in width.\textsuperscript{31} The fibers of wood pulp and MFC fibrillated from wood pulp are known to have pores. Gama et al. have defined micropores as being less than 10 nm in diameter, macropores as being more than 10 nm in diameter, and the external surface as the nonporous surface.\textsuperscript{10} According to the results of Stone et al.\textsuperscript{10} and Matsuda et al.,\textsuperscript{30} wood pulp has micropores and an external surface without macropores, whereas MFC has all three. Both St-BC and Ag-BC appear to have only micropores and external surfaces, because electron microscopy revealed no part of the structure that could

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<tr>
<th>Table 1. Physical Properties of the Cellulose Suspensions.</th>
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<tbody>
<tr>
<td>Viscosity (Pa(\cdot)s)</td>
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<tr>
<td>Ag-BC</td>
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<td>St-BC</td>
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<th>Table 2. Maximum Amounts of Adsorption of Congo red, CDH and Xyloglucan and Estimated Specific Surface Areas of Ag-BC, St-BC, MFC and LBKP.</th>
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<tr>
<td>Maximum amount of adsorption (mg/g)</td>
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<td>Congo red</td>
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<tr>
<td>Ag-BC</td>
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<td>St-BC</td>
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<td>MFC</td>
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\*Surface area of cellulose fibrils occupied by the corresponding molecule.
be categorized as having macropores.

CDH molecules are spheroidal and 4 nm × 5 nm × 18 nm in size, so they cannot be adsorbed into micropores. Therefore, CDH appears to have been adsorbed to the external surfaces of St-BC, Ag-BC and LBKP, but to the external surface and into the macropores of MFC.

The radius of gyration of the xyloglucan used in this study was about 40 nm. Therefore, xyloglucan molecules may not have been adsorbed into the micropores, but adsorbed into the macropores and to the external surfaces. Nevertheless, the specific surface areas of all four cellulose samples, derived from the xyloglucan adsorption characteristics, were larger than those derived from CDH adsorption. Whitney et al. have shown that xyloglucan cross-bridges were formed between St-BC fibrils in St-BC and xyloglucan suspensions. The cross-bridge length ranged from 20 to 50 nm, and the regions containing cross-bridges were not adsorbed to the cellulose surface. We think that such cross-bridges formed in our experiments. We assumed that the whole xyloglucan molecule had been adsorbed to the cellulose surface, so the estimated specific surface area we derived from the xyloglucan adsorption characteristics was incorrect.

The Congo red molecule is about 2.5 nm in length along its longitudinal axis, so it is smaller than the micropore diameter and would be adsorbed into the micropores. Therefore, Congo red was adsorbed into both the micropores and macropores and to the external surfaces of the cellulose samples we tested. The specific surface area of the four cellulose samples derived from Congo red adsorption differed little in value. The specific surface area derived from Congo red adsorption would thus appear to reflect the cellulose microfibril surface area because the widths of the microfibrils constituting the four cellulose samples are almost the same.

Figure 4 shows relationships between the physical properties and specific surface area estimated from Congo red, CDH and xyloglucan adsorption. CDH adsorption shows positive correlation (correlation coefficient (r) = 0.90–0.940) between the physical properties and specific surface area, whereas, with Congo red adsorption, there was no such correlation (r = 0.332–0.563). The xyloglucan adsorption data were not subjected to this analysis because the estimated specific surface area was incorrect.

There was positive linear correlation between the specific surface area derived from CDH adsorption and the viscosity, filler retention and ESI. These results suggest that these physical properties depend on the specific surface area to which CDH is adsorbed. This surface area corresponds to the external surface area of St-BC, Ag-BC and LBKP, and to the combined external surface and macropore area of MFC.

The relationships between the physical properties of the cellulose samples and their micropores were evaluated. Each micropore area was estimated by subtracting the specific surface area derived from CDH adsorption from the combined macropore and external surface area derived from Congo red adsorption. No relationship between the physical properties and the micropores for any of the four cellulose samples was apparent (data not shown).

As the viscosity, filler retention and ESI depended on the specific surface area of St-BC, Ag-BC, MFC and LBKP derived from CDH adsorption, we conclude that
the external surface area of each of these cellulose samples represents the specific surface area of constituent fibrils that are accessible to other fibrils, fillers and oil droplets. In the case of MFC, the macropore area was added to that specific surface area.

CDH molecules were only adsorbed to the external surfaces of St-BC and Ag-BC, so their specific surface areas should reflect the width of their fibrils. Ag-BC had the largest external surface area and finest fibrils of the four cellulose samples (Table 2 and Fig. 1). Therefore, the fine fibrils of Ag-BC accounted for its superior physical properties in suspension.

Although, Congo red, CDH and xyloglucan were specifically adsorbed to the surface of the cellulose molecule, this adsorption is thought to depend on the crystallinity of the cellulose molecule. It has been reported that Congo red and xyloglucan were adsorbed to the crystalline region of a cellulose sample, while CDH was adsorbed to the non-crystalline region of the cellulose sample.13,16,18 There has been little study on the crystallinity of the surface of various cellulose fibrils, so we have assumed that the crystallinity of the surface of cellulose fibrils is almost uniform. Nevertheless, it is possible that a difference in the specific surface area would reflect not only the pore structure and width of the cellulose fibrils, but also the crystallinity of the surface of the cellulose fibrils. It is necessary to evaluate the crystallinity of the surface of cellulose fibrils to elucidate this.

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References