Quaternary Ammonium Bacterial Cellulose for Adsorption of Proteins

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Bacterial cellulose (BC) has attracted attention for preparing advanced materials due to its microfibrinous structure. In this study, quaternary ammonium bacterial cellulose (QABC) as well as quaternary ammonium plant cellulose (QAPC) as adsorbents for proteins have been prepared. Introduction of the quaternary ammonium group was conducted under different conditions to determine the best conditions for modification. The fibrous structures of QABC and QAPC were quite different and determined by the starting material. The adsorption capacities of hemoglobin and an anionic dye, thymol blue, on the adsorbents QABC and QAPC which were prepared under different conditions have been investigated. QABC showed a higher adsorption capacity for hemoglobin compared with QAPC, but a lower adsorption capacity for thymol blue.

1. Introduction

Natural polysaccharides such as cellulose have been used in various fields to prepare functional materials because of their unique properties: nontoxicity, biocompatibility, biodegradability, hydrophilicity, and adsorption [1–4]. Recently, cellulose produced by bacteria has attracted attention for its potential in the preparation of advanced materials. Some bacteria, represented by *Acetobacter xylinum*, synthesize extracellular cellulose called bacterial cellulose (abbreviated as BC) [5–9]. The molecular structure of BC is an unbranched polymer of β -1, 4-linked glucopyranose residues, which is similar to that of higher plant cellulose (PC). However, the fibrous structure of BC is different from that of PC. BC is composed of microfibrils, which have a ribbon-like structure, and the thickness (0.1×10^{-6} m) is much smaller than that of plant cellulose (10×10^{-6} m). In addition, the microfibrils retain a fine network structure. As BC has various advantageous properties, such as high tensile strength, elasticity, and a large surface area, various

studies for preparing novel industrial materials from BC have been conducted [10-13]. For example, a DNA separation medium has been prepared using bacterial cellulose fibrils [14].

BC is expected to act as an adsorptive material for various species. BC exhibits a larger adsorption capacity for cellobiose dehydrogenase, which is a protein showing an affinity to cellulose, compared to wood pulp cellulose [15]. Recently, the adsorption behavior of Sb(III) on BC was compared to that of commonly used PC [16]. The initial rate of Sb(III) adsorption on BC was faster than that on PC and was probably due to the large specific surface area. On the other hand, Wang and coworkers have developed various chemically modified BCs for the adsorption of metal ions. Carboxymethylated BC for adsorption of metal ions was synthesized by *Acetobacter xylinum* by adding water-soluble carboxymethylated cellulose (CMC) to the culture medium [17]. Diethylenetriamine BC and amidoximated BC have also been prepared for the adsorptive removal of Cu(II) and Pb(II) and the adsorption kinetics were studied [18, 19]. The authors have also developed chemically modified BC as new adsorbents for metal ions and biomolecules such as proteins. Phosphorylation of bacterial cellulose (abbreviated as PBC) was found to be easier compared to that of cellulose powder originating from plant cellulose [20]. Furthermore, adsorption of cationic protein onto PBC was also examined [21]. PBC exhibited a large adsorption capacity for lysozyme compared to phosphorylated plant cellulose, which has a similar amount of phosphoric acid groups. From this precedent, chemically modified BC is expected to adsorb large quantities of proteins due to its microfibrillar structure.

In the present study, quaternary ammonium bacterial cellulose (QABC) was prepared from BC for adsorption of proteins. The cationic adsorbent was expected to show a high adsorption capacity for proteins under basic condition. Preparation of QABC was conducted under different conditions by varying basic reagents and solvents, in order to study the best conditions for modification. Adsorption behaviors of proteins and an anionic dye on QABC as well as on quaternary ammonium plant cellulose (QAPC) which was prepared under different conditions, were investigated. The adsorption experiments help in the understanding of the effect of the fibrous structure of the cellulosic adsorbents on protein adsorption.

2. Experimental

2.1 Reagents

The starting material BC was prepared from “nata de coco” by grinding, washing with distilled water, and lyophilization. Cellulose powder as PC originating from a plant source was purchased from Advantec Toyo Kaisha, Ltd., Japan. The cellulose powder is made from high purity cotton cellulose which was acid treated to remove ash. Thymol blue (Fig.1) was purchased from Tokyo Kasei, Co. Ltd., Japan. All other reagents were of reagent grade and were used as received.

Fig. 1 Molecular structure of thymol blue.

2.2 Preparation of quaternary ammonium bacterial cellulose

The dry BC was grounded and sieved to less than 200 μm . Preparation of QABC (Fig. 2) was examined under various conditions in order to determine the best conditions for modification. A typical procedure was as follows: 2.0 g of BC powder was soaked in 100 cm^3 of N,N-dimethylformamide (DMF) along with 50 cm^3 of ethylenediamine (EDA) (or triethelitetramine (TETA)). After the mixture was stirred for 24 hour, 50 g of (3-chloro-2-hydroxypropyl)-trimethylammonium chloride (QA) was added. The mixture was stirred at 60°C for 1 hour, 50 g of (3-chloro-2-hydroxypropyl)-trimethyl ammonium chloride was added again. The mixture was stirred at 80 °C for 2 hours. Subsequently, the mixture was stirred at 110°C for 24 hours. After cooling, the mixture was filtered and washed with a mixture of distilled water and 25 % ethanol, followed by 0.1 mol dm^{-3} dilute hydrochloric acid, 0.3 mol dm^{-3} dilute aqueous sodium hydroxide and finally distilled water. The product was lyophilized and ground. After sieving to less than 200 μm , 1.6 g of QABC was obtained as a white powder. The quaternary ammonium plant cellulose (abbreviated as QAPC) was prepared from PC in a similar manner to QABC, to provide a reference for QABC. QABC and QAPC examined using a scanning electron microscope (HITACHI S-5200) to evaluate the fibrous structure. The specific surface areas of QABC, QAPC, BC, and PC were determined by the N_2 -BET method using a volumetric adsorption measurement instrument (BEL Japan BELSORP mini). Furthermore, X-ray diffraction (XRD) of QABC, QAPC, BC, and PC was carried out with an X-ray diffractometer (RIGAKU RINT-2000), to evaluate the crystallinity of each cellulosic material.

Fig. 2 Synthetic route for QABC.

2.3 Adsorption tests

Adsorption experiments for hemoglobin were carried out batchwise as follows: an aqueous solution was prepared by dissolving 3.1-31.0 $\mu\text{mol dm}^{-3}$ of hemoglobin and the pH was adjusted to 9.0 using 10 mmol dm^{-3} of sodium phosphate buffer solution. A portion (15 cm^3) of the aqueous solution and QABC as the adsorbent (20mg) were mixed in a stoppered glass tube and vigorously shaken in a thermostat-regulated shaker at 30°C. After 20 hours the adsorption had reached equilibrium and the mixture was filtered. The concentration of hemoglobin in the filtrate was determined using a UV-VIS spectrophotometer (JASCO v-660).

From the concentration of hemoglobin before and after adsorption (C_0 and C_e [$\mu\text{mol dm}^{-3}$]), the

amount of metal adsorbed (q) was calculated according to the following equation (1);

$$q = \frac{C_0 - C_e}{W} \times V \quad [\mu\text{mol g}^{-1}] \quad (1)$$

where W is the dry mass of adsorbent in grams and V is the volume of solution in dm^3 .

Adsorption of thymol blue on QABC and QAPC was also examined in a similar manner, using a portion (15 cm^3) of an aqueous solution containing $0.43\text{--}2.14 \text{ mmol dm}^{-3}$ of thymol blue and 20 mg of the adsorbent. The concentration of thymol blue in the filtrate was determined using a UV-VIS spectrophotometer (JASCO v-660). Furthermore, adsorption experiments for hemoglobin (at pH 9), myoglobin (at pH 9), thymol blue, 1,10-phenanthroline, As(III) (at pH 13), and Sb(III) (at pH 13) using 20 mg of adsorbent (QABC, QAPC, BC, and PC) were also carried out, in order to study the adsorption properties of each adsorbent. The concentrations of As(III) and Sb(III) before and after adsorption were analyzed using an ICP/AES spectrometer (Shimadzu ICPS-7000).

3. Results and Discussion

3.1 Characteristics of quaternary ammonium bacterial cellulose

The scanning electron micrographs of QABC and QAPC are shown in Fig. 3. As shown, the surface structure is quite different from that of QAPC from plant cellulose: a microfibrillar ribbon structure can be seen on the surface of the QABC fibers. As the microfibrillar structure of QABC is similar to that of the starting material BC, the procedure for introducing quaternary ammonium groups under the experimental conditions does not influence the microfibrillar structure. The specific surface areas of cellulose adsorbents calculated using the N_2 -BET method were $15.5 \text{ m}^2 \text{ g}^{-1}$ for QABC, $1.8 \text{ m}^2 \text{ g}^{-1}$ for QAPC, $21.8 \text{ m}^2 \text{ g}^{-1}$ for BC, and $1.1 \text{ m}^2 \text{ g}^{-1}$ for PC, respectively. The specific surface area of QABC is thus much larger compared to that of QAPC. Also, the crystallinities of QABC, QAPC, BC, and PC were evaluated using X-ray diffractometer. The crystal structure of QABC was partly destroyed compared with that of the starting material BC. By contrast, the crystal structure of QAPC was mostly retained after introduction of the quaternary ammonium group.

Fig. 3 Scanning electron micrographs of QABC (left) and QAPC (right) at $\times 10,000$ magnification.

3.2 Adsorption of hemoglobin on quaternary ammonium bacterial cellulose

The dependence of pH for the adsorption of hemoglobin onto BC and QABC is shown in Fig. 4. The adsorption of hemoglobin on BC was quite small. By contrast, adsorption of hemoglobin on QABC, in which a cationic quaternary ammonium group was introduced, increased with increasing pH and reached a maximum value under basic conditions. As the isoelectric point of hemoglobin is about 6.8, hemoglobin exists as an anionic species under basic conditions. Thus QABC was found to act as a cationic adsorbent which captures proteins such as hemoglobin via electrostatic interaction.

Fig. 4 Adsorption profiles for hemoglobin onto BC and QABC as a function of pH: adsorbent; 10mg, volume; 15cm³.

3.3 Adsorption capacity of quaternary ammonium bacterial cellulose for hemoglobin

Figure 5 shows adsorption isotherms for hemoglobin onto QABC and QAPC, which were prepared under the same conditions from BC and PC, respectively. As the amounts of adsorption onto QABC and QAPC were quite different, it is likely that the amount of the quaternary ammonium groups introduced

Fig. 5 Adsorption isotherms for hemoglobin on QABC (No.2 in Table 1) and QAPC (No.8 in Table 1) at 30°C. pH_{ini} 9.0, adsorbent, 20 mg, volume=15cm³, [hemoglobin]_{ini}=0.2-2.0 g dm⁻³.

Fig. 6 Adsorption isotherms for hemoglobin on QABC adsorbents (No.2-6 in Table 1) at 30°C. pH_{ini} 9.0, adsorbent, 20 mg, volume=15 cm³, [hemoglobin]_{ini} = 0.2-2.0 g dm⁻³.

on QABC is larger than that for QAPC prepared under the same conditions. Figure 6 shows adsorption isotherms for hemoglobin onto QABCs, which are prepared under different conditions. The amount of adsorption of hemoglobin on QABCs increases with increasing hemoglobin concentration. The equilibrium experimental data were correlated with the Langmuir isotherm model in order to determine the maximum adsorption capacity of hemoglobin on QABC (q_{max}). The Langmuir model assumes monolayer adsorption as shown in the following equation (2):

$$\frac{C_e}{q} = \frac{C_e}{q_{max}} + \frac{1}{q_{max} K_L} \quad (2)$$

Table 1 Reactive conditions for preparing adsorbents (QABC; No. 1-7, QAPC; No. 8-13) and the maximum adsorption capacities for hemoglobin and thymol blue on each adsorbent.

No.	Conditions of preparation of QABC and QAPC*					q_{max} for Thymol	q_{max} for Hemoglobin
	base	Amount of base	Amount of QA	Solvent	Starting material	Blue [μ mol g ⁻¹]	[μ mol g ⁻¹]
1	EDA	50 [cm ³]	75 [g]	DMF	BC	-	5.07
2	EDA	50 [cm ³]	25 [g]	DMF	BC	2.62	1.08
3	EDA	50 [cm ³]	25 [g]	DMSO	BC	2.72	1.00
4	EDA	50 [cm ³]	25 [g]	Pyridine	BC	1.66	0.073
5	EDA	50 [cm ³]	25 [g]	Methanol	BC	1.36	0.841
6	EDA	50 [cm ³]	25 [g]	2-propanol	BC	0.837	0.315
7	TETA	50 [cm ³]	25 [g]	DMF	BC	0.272	0.642
8	EDA	50 [cm ³]	25 [g]	DMF	PC	12.8	0.348
9	EDA	50 [cm ³]	25 [g]	DMSO	PC	16.5	0.071
10	EDA	50 [cm ³]	25 [g]	Pyridine	PC	12.8	0.062
11	EDA	50 [cm ³]	25 [g]	2-propanol	PC	33	0.546
12	NaOH	50 [cm ³]	25 [g]	Methanol	PC	41.2	0.753
13	urea	50 [g]	25 [g]	DMF	PC	1.73	0.105

*Common conditions are as follows: amount of cellulose, 0.3 g; solvent, 30 cm³; reaction time.

where q denotes the amount of hemoglobin adsorbed on QABC [$\mu\text{mol g}^{-1}$], C_e denotes the equilibrium concentration of hemoglobin in the aqueous solution, and K_L denotes the adsorption equilibrium constant [$\text{L } \mu\text{mol}^{-1}$]. In a similar manner, the maximum adsorption capacity of thymol blue on each adsorbent was calculated from the Langmuir equation (2) for the adsorption isotherm of thymol blue. The anionic dye, thymol blue, was used as a model material as it is a smaller molecule compared with hemoglobin and should be adsorbed on cationic adsorbents via electrostatic interaction. The amount of thymol blue adsorption partly reflects the degree of substitution of the QA group onto the adsorbents. The maximum adsorption capacities of hemoglobin and thymol blue on adsorbents QABC and QAPC, as well as the reactive conditions for preparing adsorbents are summarized in Table 1. Additionally, the relationship between the adsorption capacity of thymol blue and the adsorption capacity of hemoglobin onto QABCs and QAPCs is plotted in Fig. 7. The amount of adsorption increases with increasing amounts of QA during preparation (No. 1 and 2). The solvent for the preparation of QABC is also important for effective modification (No. 2 and 6): DMF and DMSO (dimethyl sulfoxide) are more favorable for preparation of QABC. As shown in Table 1, it is apparent that QABC shows a larger adsorption capacity for hemoglobin than QAPC prepared under the same conditions. The result suggests that the degree of substitution by quaternary ammonium groups is influenced by the morphology of the cellulose, which differs according to the origin of cellulose. As the BC microfibril is much thinner than the fiber of plant cellulose, modification of BC using QA proceeds easier than that of PC, probably due to the easier accessibility for the reagents. The result is similar to that shown in the preparation of phosphorylated bacterial cellulose [20]. It should be noted that the adsorption behaviors of QABC and QAPC are different as shown in Fig. 7. For both adsorbents QABC and QAPC, the q_{max} values for hemoglobin increases with increasing q_{max} values for thymol blue. However, the maximum adsorption capacity for thymol blue on QABC is much smaller than that on QAPC, despite the larger surface area and larger adsorption capacity for hemoglobin.

Fig. 7 Relationship between the adsorption capacity for thymol blue and the adsorption capacity for hemoglobin onto QABCs (No.2-6 in Table 1) and QAPCs (No.8-13 in Table 1).

In order to study adsorption properties of each adsorbent, adsorption tests for various materials, which are different in molecular weight, were carried out. Sb(III) is found to be adsorbed on cellulosic materials [16, 22]. Thymol blue is a hydrophobic material and is present in an anionic state. 1,10-Phenanthroline is also a hydrophobic material but is not present in an anionic state. The adsorption behaviors of such materials would provide information on the important factors for adsorption on the adsorbents, such as size, hydrophobicity, and ionicity. Table 2 shows the adsorption percentages of various materials on QABC, QAPC, BC, and PC. QABC exhibited a much higher adsorption percentage for the proteins hemoglobin and myoglobin compared with QAPC, BC, and PC. Sb(III) and As(III) were also adsorbed on each adsorbent, however, differences in adsorption percentages were not so high compared with those of the adsorption percentages for proteins. On the other hand, the adsorption percentage of thymol blue on QAPC was much higher than that on QABC. However, the starting material BC exhibits a much higher adsorption for thymol blue as well as 1,10-phenanthroline, compared with PC. The results suggest that the surface properties of QABC, especially hydrophobicity, were altered from that of the original material BC by the chemical modification. On the basis of these results, QABC prepared from bacterial cellulose appears to be a favorable adsorbent for proteins compared with QAPC prepared from plant cellulose.

Table 2 Adsorption of various materials on QABC, QAPC, BC, and PC*.

Adsorbent	material	hemoglobin	myoglobin	thymol blue	1,10-phenanthroline	As(III)	Sb(III)
	M.W. (A.W.)	64,500	17,200	466	180	(74.9)	(121.7)
	initial conc.	6.20 [$\mu\text{mol dm}^{-3}$]	23.3 [$\mu\text{mol dm}^{-3}$]	0.22 [mmol dm^{-3}]	0.50 [mmol dm^{-3}]	0.12 [mmol dm^{-3}]	0.12 [mmol dm^{-3}]
QABC	q [mmol g^{-1}]	88.0×10^{-3}	423×10^{-3}	0.249	0	0.456	2.87
QAPC		3.88×10^{-3}	22.8×10^{-3}	3.45	0.013	0.320	1.65
BC		0.68×10^{-3}	9.32×10^{-3}	3.25	0.22	0.457	2.09
PC		0.53×10^{-3}	6.87×10^{-3}	0	0	0.566	1.88

*Common conditions are as follows: adsorbent, 20 mg, volume=15cm³.

4. Conclusions

In the present study, quaternary ammonium groups were introduced to bacterial cellulose in order to develop a new absorbent for proteins. The microfibrinous structure of the modified bacterial cellulose was mostly retained after chemical modification. A model protein, hemoglobin, was adsorbed on the quaternary ammonium bacterial cellulose under basic conditions via electrostatic interaction. The adsorption capacity for hemoglobin on quaternary ammonium bacterial cellulose is higher than that on quaternary ammonium plant cellulose which was prepared under the same conditions. Quaternary ammonium bacterial cellulose exhibited the selectivity for proteins over other materials. Therefore,

quaternary ammonium bacterial cellulose is proposed as a new adsorptive material for relatively large biomolecules on the basis of its large surface area.

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