Structure of Nascent Microbial Cellulose IV. Influence of Size and Substitution Groups of Direct Dye on Nascent Microbial Cellulose

Hiroyasu KIDO[†] and Akira KAI

Department of Industrial Chemistry, Tokyo Metropolitan University,
Minami-Ohsawa 1-1, Hachioji-shi, Tokyo 192-0397, Japan

(Received December 3, 1997)

ABSTRACT: The structures of products obtained from *Acetobacter*-cultures in the presence of direct dyes and cellulose regenerated from those were examined by X-ray measurements. The products obtained in the presence of direct dyes showed the X-ray diffraction diagrams with two distinct peaks different from the diffraction diagram of microbial cellulose. These products were crystalline complexes containing the direct dye between the cellulose sheets corresponding to the (1 $\bar{1}$ 0) plane of microbial cellulose. Cellulose I was regenerated from the Direct Brown 106 and the Direct Blue 71 complexes, cellulose IV was regenerated from the Direct Red 28 complex and cellulose II was regenerated from the Direct Red 80 complex. The different effects of direct dyes on the structure of the nascent cellulose may be due mainly to the number of sulfonate groups per unit of molecular weight.

KEY WORDS *Acetobacter xylinum* / Microbial Cellulose / Direct Dye / Dye-Cellulose Complex / Cellulose I / Cellulose II / Cellulose IV /

Acetobacter xylinum (*A. xylinum*) produces extracellular cellulose I α rich cellulose.¹ However, this nascent cellulose is in a noncrystalline state.² Accordingly, various substances, such as medium components,³ heavy metal salts,⁴ and the direct dye for cellulose^{5–8} diffuse into the nascent cellulose. Therefore, microbial cellulose (MC) with different fine structures can be prepared from interactions of the diffusion substance and nascent cellulose according to characteristics of the diffusion substance.

The crystalline complex composed of a dye and cellulose, obtained from *A. xylinum*-culture in the presence of Fluorescent Brightener, is transformed into cellulose I by dye-extraction.^{5–10} This cellulose is not a cellulose I α rich structure for ordinary MC but cellulose I β rich structure.¹¹ Moreover, cellulose IV is regenerated from the complex produced in the presence of Direct Red 28.^{12,13} Cellulose II is regenerated from complexes produced in the presence of Direct Blue 1, 14, 15, or 53^{13–15} having the same biphenylenebis(azo) skeletal structure as Direct Red 28, Direct Red 75 or 79¹⁶ having ureylenebis(naphthylazo)benzene skeletal structure. But, there are slight differences in the structures of these regenerated celluloses II. Although the structures of regenerated celluloses depend on the structures and properties of dyes, the main factor is not always clear.

This paper reports the influence of dyes on the structure of nascent MC, judging from structures of the products and regenerated celluloses obtained by *A. xylinum*-cultures in the presence of various dyes. Such dyes are Direct Brown 106 (DBr106) with the longest molecular length in a direct dye, Direct Red 28 (DR28) with the shortest molecular length, Direct Red 80 (DR80) with a molecular weight somewhat larger than DBr106 and having two more sulfonate groups than DBr106 or Direct Blue 71 (DB71).

EXPERIMENTAL

Preparation of Samples

Product Sample. Cultivation was performed by the methods in previous papers.^{14–16} *A. xylinum* (IFO 13693) was cultured in a 15 cm diameter petridish containing 110 ml Hestrin–Schramm medium¹⁷ (pH 6.8) at 28.0°C for 3 days in an incubator. 60 ml of cellulose-free cell suspension, prepared from matured petridish in 120 ml phosphate buffer solution, were added to 140 ml of HS medium (pH 7.0) containing a direct dye of 0.1 wt% and the mixture was incubated at 28.0°C for 24 h under static conditions. The product was collected by centrifugation (6000 rpm, 5.15 g, 20 min) and washed well with 0.1 wt% aqueous NaOH solution to remove the dye not related to dyeing and then washed thoroughly with distilled water until alkali-free. These products were preserved at about 3°C in a wet state.

The direct dyes used in this work are DB71 (tetrasodium 2-{4'-[4''-(6'''-amino-1'''-hydroxy-3'''-sulfonato-2'''-naphthylazo)-6''-sulfonato-1''-naphthylazo]-1'-naphthylazo}-naphthalene-4,8-disulfonate, Sumitomo Chemicals), DBr106 (tetrasodium *N,N'*-bis-{3-methyl-4-{3'-methyl-4'-[2''-sulfonato-4''-(4'''-sulfonatophenylazo)phenylazo]phenylazo}phenyl}urea, Sumitomo Chemicals), DR28 (2,2'-[4,4'-biphenylenebis(azo)] bis[1-amino-naphthalene-4-sulfonic acid]disodium salt, Aldrich Chemical), DR80 (hexasodium *N,N'*-bis{1-hydroxy-2-[2'-sulfonato-4'-(4''-sulfonatophenylazo)phenylazo]-3-sulfonato-6-naphthyl}urea, Ciba-Gaigy). The chemical structures of these dyes are shown in Figure 1.

DB71 and DR28 were purified by salting out. 1 wt% aqueous solution of a dye was heated at 80°C and filtered to reject insoluble matter. 40 wt% aqueous sodium acetate solution in a ratio of 1 to 5 was added to the filtrate with agitation at 80°C and settled until it reaches to the room temperature. The sedimental dye was

[†] Present address: Mitsui Toatsu Chemicals Inc., Kasumigaseki 3-2-5, Chiyoda-ku, Tokyo 100-0013, Japan.

Direct Brown 106

Direct Blue 71

Direct Red 28

Direct Red 80

Figure 1. Chemical structures of direct dyes.

collected on the glass filter and washed with hot ethanol until it disappears. The dye was refined by repeating the operation three times and dried over P_2O_5 under reduced pressure. DBr106 and DR80 were purified by recrystallization. The dye was added to the mixed solvent of ethanol and water (in case of DBr106, EtOH:H₂O=90:10, in case of DR80, EtOH:H₂O=70:30) and dissolved at 70°C and filtered. The filtrate was left in static condition to recrystallize the dye. The dye was refined by repeating the operation three times and dried over P_2O_5 under reduced pressure.

Regenerated Cellulose Sample. The dye-extraction of the above products was performed by boiling in 70 vol% ethanol solution for 18 h and fresh aqueous ethanol solution was exchanged every 3 h. The dye-extracted sample was again boiled in 1.0 wt% aqueous sodium hydroxide solution for 10 h under N₂ atmosphere. The regenerated cellulose sample was neutralized with 1.0 vol% aqueous acetic acid and rinsed well with distilled water. The sample was preserved as above.

Methods for Measurement

X-Ray Measurement. This was done as in previous papers.^{14–16} The X-ray diffraction diagram of an uniplanar oriented membrane of the product was measured by reflection and transmission. The membranes were prepared from the preserved product, which was dried on a Teflon plate at atmospheric conditions. The diffraction diagrams of an isotropic product and re-

generated cellulose were made by transmission. In this method, the sample was compressed as a pellet into the pore of a sample holder of 2 mm in diameter and 1 mm in thickness. The X-ray diffractogram was measured by a MXP¹⁸ diffractometer (MAC Science) with Ni-filtered Cu-K_α radiation. Measurement conditions were as follows: divergence slit, 1.0 mm; receiving slit, 0.15 mm; scanning speed, 4.0° min⁻¹; X-ray radiation, 40 kV, 200 mA.

RESULTS AND DISCUSSION

Figure 2 shows X-ray diffractograms of isotropic MC and the isotropic product obtained from *A. xylinum*-culture in the presence of DB71, DBr106, DR28, or DR80 (DB71 product, DBr106 product, DR28 product, and DR80 product). 2θs of diffraction peaks of each diagram are listed in Table I.

In all cases, the products obtained in the presence of a direct dye showed characteristic diagrams with two diffraction peaks: one occurs strongly at 20–23° and the others weakly at 9° to 17°. The diffractogram of the DR80 product was somewhat different from the other three diagrams because the peak at high angle side occurs near 20°. The diffractogram of the DR80 product is similar to the diagrams of the DB1, 14, 15, 53, DR75, and 79 products as reported previously.^{14–16} These four diffractograms of the product having two peaks, one at high angle side and the other at low angle side, are similar

Figure 2. X-Ray diffractograms of microbial cellulose and product samples from *Acetobacter*-culture in the presence of a dye of 0.1 wt%. a, MC; b, DB71 product; c, DBr106 product; d, DR28 product; e, DR80 product.

Table I. X-Ray diffraction angles of MC and products obtained from *Acetobacter*-culture in the presence of a direct dye

Sample	2θ/deg			
MC		14.5 (w)	16.8 (w)	20.4 (w) 22.7 (s)
DB71			16.8 (w)	23.5 (s)
DBr106	9.0 (vw)	10.8 (vw)		23.0 (s)
DR28		11.0 (w)		23.0 (s)
DR80	9.0 (vw)	12.0 (vw)		20.6 (s)

to those of products obtained in the presence of various direct dyes.^{8,12,14-16} The diffractogram of the product suggests that all direct dye diffuse into nascent MC and form the characteristic complex composed of a dye and cellulose instead of the ordinary structure of MC.

The diffractograms of uniplanar-orientated membranes of DBr106, DB71, DR28, and 80 products measured by transmission and reflection methods are shown in the Figure 3. 2θs of the diffraction angle of each product are listed in Table II.

When the DB71 product membrane is measured by reflection, a strong and a weak diffraction peaks occur at 8.6° and 16.6°, respectively, whereas, in case of transmission, strong and weak peaks occur at 23.5° and 16.7°, respectively. This shows that the diffraction plane near 16.7° does not attain uniplanar-orientation. In the reflection diffractogram of the DBr106 product membrane, strong and very weak peaks occur at 10.8° and 22.5°, whereas, in transmission, strong and weak peaks occur at 23.0° and 10.5°, respectively. Similar uniplanar-orientation was observed in the case of the DR28 product

Figure 3. X-Ray diffractograms of oriented membrane of product samples measured by transmission (T) and reflection (R). a, DB71 product; b, DBr106 product; c, DR28 product; d, DR80 product.

Table II. 2θ values of diffraction peaks in diffractograms of oriented membrane of the products measured by transmission and reflection methods

Dye	2θ/deg			
DB71	R	8.6 (s)	16.6 (w)	
	T	—	16.7 (w)	23.5 (s)
DBr106	R		10.8 (s)	
	T		10.5 (vw)	23.0 (s)
DR28	R		11.2 (s)	23.0 (w)
	T		10.7 (w)	23.0 (s)
DR80	R	9.0 (vw)	12.4 (w)	21.9 (s)
	T	9.0 (vw)	—	20.0 (s)

membrane. The diffractograms of three products resemble that of the product obtained in the presence of Fluorescent Brightener as reported previously.⁸ In contrast, in both the reflection and transmission diffractograms of the DR80 product, strong and weak peaks occur near 21° and 9.0°, respectively. The diffractogram of the DR80 product resembles those of DR75 and 79 products.¹⁶

The diffraction plane at the low angle side of DB71, DBr106, or DR28 product membrane parallels the surface of the membrane, whereas the diffraction plane at the high angle side is perpendicular to the surface of the membrane. The behavior of the uniplanar orientation of these product membranes is similar to that of the

Figure 4. X-Ray diffractograms of cellulose regenerated from product samples. a, DB71 cellulose; b, DBr106 cellulose; c, DR28 cellulose; d, DR80 cellulose.

Table III. X-Ray diffraction angles of cellulose I, II, IV_{II}, and cellulose regenerated from product samples

Sample	2θ/deg		
	(110)	(110)	(020)
MC	14.5	16.8	22.7
DB71	14.5	16.3	22.6
DBr106	15.1	16.3	22.4
Cellulose IV _{II}	15.4	15.4	22.4
DR28	15.4	15.4	22.4
Cellulose II	12.0	19.8	21.1
DR80	12.2	20.4	21.7

product membrane obtained in the presence of Fluorescent Brightener.⁸ The strong diffraction peak at the low angle side suggests that spacing is due to the inclusion of a dye between the cellulose sheets corresponding to the (110) plane of MC. The DR80 product membrane does not show such uniplaner-orientation. This is due to the removal of dye from the complex during rinsing of the product sample.

Figure 4 shows the X-ray diffractograms of celluloses (DB71 cellulose, DBr106 cellulose, DR28 cellulose, and DR80 cellulose) regenerated from the product by dye-extraction. 2θs of each diffraction peak are listed in Table III.

As apparent from Figure 4 and Table III, the diffractograms of DB71 and DBr106 celluloses are almost the same as that of cellulose I. The diagram of the DR28 cellulose coincides with that of cellulose IV. The diagram of DR80 cellulose is similar to that of cellulose II. According to the characteristics of each direct dye, the regenerated cellulose forms specific crystals.

The fine structure of regenerated cellulose seems to be produced in the process of sorption and desorption of a direct dye. The degree of interaction between a direct

Table IV. Molecular weights of dyes

Dye	MW	NH	NS	MW/N	Crystal type	Ref
FB	916	8	2	458	Cellulose I	7
DBr106	1198	2	4	300	Cellulose I	^a
DB71	941	2	4	235	Cellulose I	^a
DR28	812	2	2	406	Cellulose IV _I	13 ^a
DR79	948	4	4	237	Cellulose II	17
DR75	902	6	4	226	Cellulose II	17
DB1	904	4	4	226	Cellulose II	15
DB14	872	4	4	218	Cellulose II	16
DR80	1236	4	6	206	Cellulose II	^a

NS: Number of sulfonate, NH: Number of group with hydrogen bonding ability, MW: Molecular weight, N: Number. ^a Present work.

dye and cellulose chain depends on the planarity of a dye molecule, straightness, length of the conjugated double bond, proton donor or acceptor.¹⁸ Although there are no differences in the planarity and straightness of four dyes, the length of conjugated double bond, groups having the amount of the hydrogen bonding ability, the number of sulfonate group differs. The order of length of the conjugated double bond is DBr106 > DR80 > DB71 > DR28. In case of the number of the group having the hydrogen bonding ability, DR80 has four, DR28, DB71, and DBr106 have two except for the sulfonate group, respectively. In case of the sulfonate group, DR80 has six, DBr106 and DBu71 have four, and DR28 has two.

As cellulose I is regenerated from DBr106 and DB71 products, it seems that these dyes do not have influence on conformation of cellulose chains. However, cellulose regenerated from the complex including a dye between the cellulose sheets does not form cellulose I_α rich but forms cellulose I_β rich crystal.¹¹ This suggests that these dyes influence only the arrangement of cellulose chains. Similarly, that cellulose IV_I is regenerated from the DR28 product¹³ indicates the conformation of cellulose chains does not change,¹⁹ but the arrangement changes. DR80 influences conformation and arrangement of cellulose chains during regeneration and hence, regenerated cellulose is cellulose II.

The length of the conjugated double bond and number of groups having hydrogen bonding ability may not be the factor determining the crystal type of regenerated cellulose. Although the number of sulfonate group in a dye is not concerned apparently, it is assumed that the molecular weight per sulfonate group has great influence on crystal type (Table IV). Cellulose II is regenerated from the product obtained in the presence of a direct dye with molecular weight less than 300 per sulfonate group and in case of more than 300, cellulose I or IV_I is regenerated. In the case of DB71, the result is exceptional.

Although the affinity of the direct dye toward cellulose might be determined by various factors, the influence of the sulfonate group on the conformation of cellulose chains seems predominant.

REFERENCES

1. F. Horii, A. Hirai, and R. Kitamaru, *Macromolecules*, **20**, 2117 (1987).
2. A. Kai and T. Koseki, *Makromol. Chem.*, **186**, 2609 (1985).

3. J. R. Colvin, M. Takai, L. C. Sowden, and J. Hayashi, *Int. J. Biol. Macromol.*, **4**, 244 (1982).
4. A. Kai and J. Kogusuri, *Nippon Kagaku Kaishi*, 198 (1982).
5. A. Kai, *Makromol. Chem., Rapid Commun.*, **5**, 307 (1984).
6. A. Kai, *Makromol. Chem., Rapid Commun.*, **5**, 653 (1984).
7. A. Kai and P. Xu, *Polym. J.*, **22**, 955 (1990).
8. A. Kai and P. Xu, *Kobunshi Ronbunshu*, **48**, 449 (1990).
9. C. H. Haigler, R. M. Brown, and M. Benziman, *Science*, **210**, 903 (1980).
10. M. Benziman, C. H. Haigler, A. R. White, and K. M. Copper, *Proc. Natl. Acad. U.S.A.*, **77**, 6678 (1980).
11. A. Kai, P. Xu, F. Horii, and S. Hu, *Polymer*, **35**, 75 (1994).
12. A. Kai, Y. Kido, and N. Ishida, *Chem. Lett.*, 949 (1990).
13. A. Kai and Md. I. H. Mondal, *Int. J. Biol. Macromol.*, **20**, 221 (1997).
14. Md. I. H. Mondal and A. Kai, *Polym. J.*, **30**, 78 (1998).
15. Md. I. H. Mondal and A. Kai, *Polym. J.*, **30**, 84 (1998).
16. A. Kai and Md. I. H. Mondal, *Polym. J.*, **30**, 398 (1998).
17. S. Hestrin and M. Schramm, *Biochem. J.*, **58**, 345 (1954).
18. N. Kuroki, in "Theoretical Chemistry of Dyeing," Maki-shoten, Tokyo, 1966, p 149.
19. J. Hayashi, H. Kon, M. Takai, M. Hatano, and T. Nozawa, in "The Structures of Cellulose," R. H. Atalla, Ed., ACS Symposium Series 340, Washington, D.C., 1985, p 135.