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A facile method of determination for distribution of the substituent in *O*-methylcelluloses using ^{1}H-NMR spectroscopy

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Summary

A facile method to determine the distribution of methyl groups in regioselectively methylated celluloses on anhydroglucose unit was developed using solution ^{1}H-NMR analysis. The determination was deduced from the assignment of the signals of each proton, which directly attached to the glucopyranose ring carbon for partially methylated 2,3-di-*O*-methylcellulose samples observed in D_2O. These data for the distribution of the methyl groups corresponded to those based on the gas-chromatographic analysis. This method using the ^{1}H-NMR spectroscopy can facilitate the measurements of the distribution of methyl groups for randomly substituted and commercially available *O*-methylcelluloses.

Introduction

Methods to measure the distribution of substituents within the anhydroglucose units of cellulose derivatives have been developed although it has not been found yet definite methods to determine the distribution of substituents along the molecular chain [1].
The first analytical method was based on the gas- or liquid-chromatographic analysis of partially methylated alditol acetates [2-4] derived by acetylation of hydrolyzed and reduced cellulose derivatives. Recently, Mischnick *et al*. reported the improved methods[5,6]. These methods were, however, laborious because cellulose ethers had to be converted to analyzable derivatives after several chemical steps.
The convenient methods to determine the distribution of substituents within the anhydroglucose units by solution ^{13}C-NMR spectroscopy were also introduced by Miyamoto *et al.*[7,8]. They proposed that the distribution of acetyl groups in cellulose acetate was determined by considering that the acetyl carbonyl carbon ^{13}C-NMR signals of acetylated samples were split into a triplet corresponding to 2, 3, and 6 positions on the anhydroglucose units. Recently, Tezuka *et al*.[9,10] reported a method to determine the methyl group distribution of *O*-methylcelluloses using ^{13}C-NMR spectroscopy after acetylation of the unsubstituted hydroxyl groups in the

O-methylcelluloses.
To date, we have investigated the relationship between the hydrogen bonding formation and the physicochemical properties in cellulose derivatives using regioselectively substituted 2,3-di-*O*-methylcellulose[11], 6-*O*-methylcellulose[12], tri-*O*-methylcellulose[13] as cellulose model compounds[14-23]. The distribution of substituents has been found to influence the hydrogen bonding formation, particularly intramolecular hydrogen bonds, which may affect gelation [18,19], liquid crystallization[20], crystallization, and chemical activity of the hydroxyl groups[15] in cellulose derivatives. It was also reported to affect the enzymatic degradation[21,22]. More recently, we also indicated that the distribution of the methyl substituent might contribute the formation of hydrogen bonds as well as the hydrophobic interaction engaging cross-linking junction for thermotropic aqueous gels of *O*-methylcelluloses[24]. Therefore, a facile method to determine precisely the distribution pattern has been eagerly desired.
If it were possible to easily determine the distribution of the methyl substituent on *O*-methylcellulose using ^{1}H-NMR, it would make the measurement time shorter with small amounts of the sample. In this article, we will propose a new convenient method to determine the distribution of the methyl substituent within the anhydroglucose units in *O*-methylcellulose using solution ^{1}H-NMR spectroscopy. It should be noted that in the present method, we have focused on the proton signals directly attached the ring carbon, not as for the hydroxyl proton.

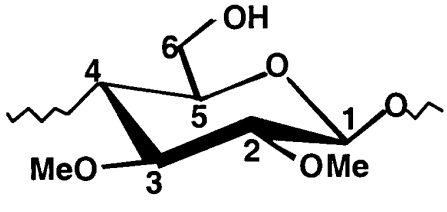
Experimental

Materials

A series of *O*-methylcellulose samples (2,3MC-n: n=1~3) which were regioselectively subsitituted only at the C-2 and C-3 positions (shown below with the numbering of the position) were prepared by multiple methylation of 6-*O*-triphenylmethylcellulose (6-TC) as a starting material basically followed by the method reported previously [11]. In the present study, the second and the third methylation were repeated exactly in the same manner of the first methylation step. The numbering of the sample corresponded to the individual methylation step; for example 2,3MC-1 indicates the sample single-methylated. The commercial *O*-methylcellulose was provided by Shin-Etsu Chemical Co. The samples used have were all dissolved in water, and the supernatant of the solution after centrifugation was dried at 50 °C to remove insoluble impurities.

Measurements

The two-dimensional-NMR spectra were obtained using a JEOL Alpha 500 spectrometer (500 MHz for ^{1}H and 125.65 MHz for ^{13}C). The pulse program of the



phase-sensitive ^{1}H-^{13}C HSQC (heteronuclear single quantum coherence) using bilinear rotation decoupling pulse were employed from the JEOL software library. The internal standard, sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (δH: 0 ppm) and the central signal of DMSO-d_6 (δC: 39.5 ppm) were used as reference for ^{1}H-^{13}C NMR spectra, respectively. ^{1}H-NMR was measured with a JEOL-GX 400 FT-NMR spectrometer (400 MHz) at 15 °C in three –solvent systems; D_2O, DMSO-d_6 and the mixture (DMSO-d_6/D_2O). The mixed solvent system was composed of DMSO-d_6 containing 1% (v/v) D_2O (DMSO-d_6/D_2O). The chemical shift (δ) in DMSO-d_6 and DMSO-d_6/D_2O was obtained relative to that of tetramethylsilane (δH: 0 ppm), while in D_2O, the chemical shift was calculated relative to that of HDO (δH: 4.8 ppm). The spectra were recorded after 400 scans for commercial *O*-methylcellulose and 40 scans for 2,3MC samples.

The gas-chromatographic analysis for the determination of the distribution of the substituent in the samples was reported in the previous paper[11,13].

Results and Discussion

In ^{1}H-NMR spectra for cellulose and its derivatives, the individual signal in the polymers is supposed to be broader than the monomer (anhydroglucose unit), because the segment of the polymer molecular chain is less flexible. Nevertheless, the proton signals which overlap with each other in the ^{1}H-NMR spectra could be identified as separated as a signal using the two-dimentional ^{1}H-^{13}C-NMR spectroscopy [25]. Figure 1 shows the two-dimentional ^{1}H-^{13}C-NMR spectra of 2,3-di-*O*-methylcellulose

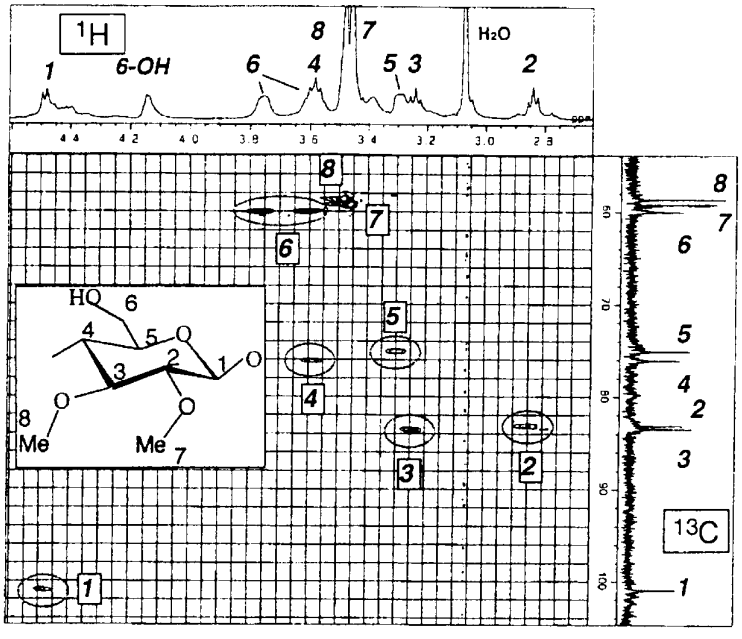


Figure 1 ^{1}H-^{13}C-NMR spectrum of 2,3-di-*O*-methylcellulose in DMSO-d_6 (containing a slight amount of D_2O).

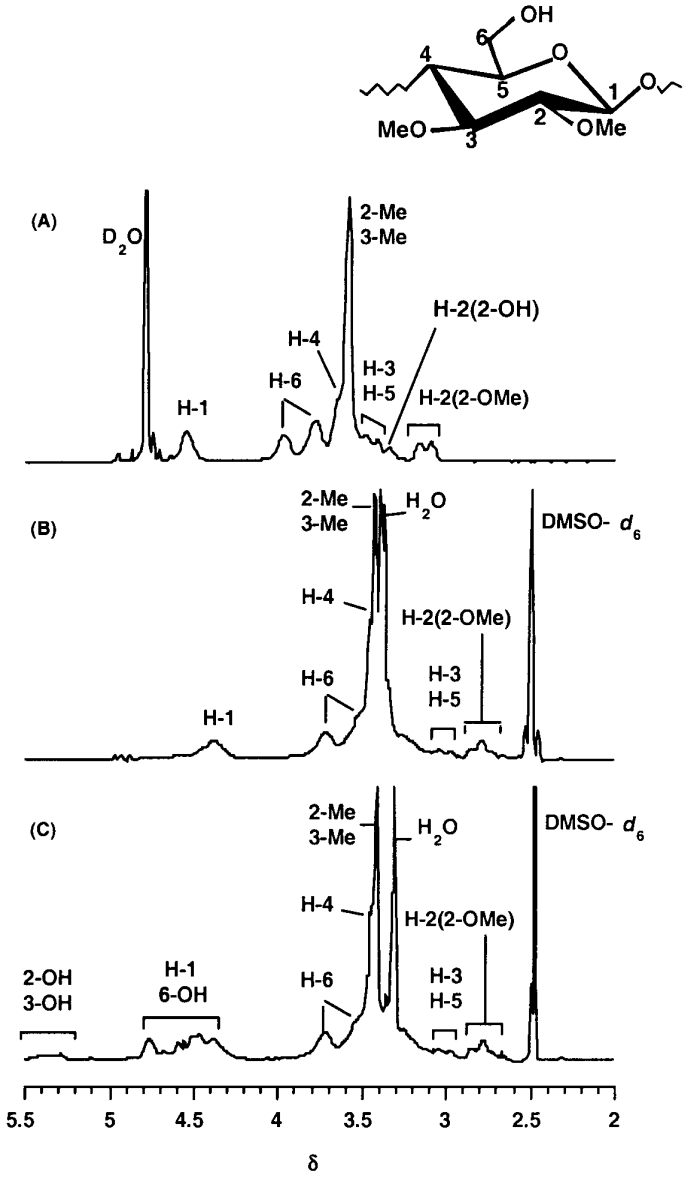


Figure 2 ^{1}H-NMR spectra of 2,3-di-*O*-methylcellulose at 15 °C: (A) in D_2O, (B) in DMSO-d_6 and D_2O (containing 1% DMSO-d_6), (C) in DMSO-d_6.

in DMSO-d_6 containing a slight amount of D_2O[26]. The signal number indicates the number of the carbon position on the anhydroglucose unit in the figure; for example the carbon signal at 1 position is labeled as C(1). The proton signals of the unsubstituted hydroxyl groups at the 2, 3 and 6 position of the carbon are labeled as 2-, 3-, 6-OH. The proton signals of methyl groups at 2, 3 and 6 position are labeled as 2-, 3-, 6-Me. The possible assignments of the carbon signals were already proposed [27]. The proton signals can be assigned using correlation between the proton signals and those of ^{13}C on two-dimensional ^{1}H-^{13}C-NMR spectra. The signal of C(1) bearing two oxygen atoms differs from those of C(2), C(3) and C(5). The signal of C(6) locates in the higher magnetic field because C(6) is in a primary alcohol group.

The solvent may also influence resolution of the proton signals attached directly to ring carbons. Therefore three-different solvent systems for ^{1}H-NMR measurements were compared as shown in Figure 2. The signals due to 2-Me and 3-Me were not well distinguished with each other in these spectra. While H-1 overlapped with that of

6-OH in DMSO-d_6 (C in Fig. 2), it became a single signal in D_2O or DMSO-d_6/D_2O. This is due to deuteration of three hydroxyl groups (2-, 3-, and 6-OH) into OD. In DMSO-d_6/D_2O and DMSO-d_6 (B and C in Fig. 2), the signals (3.3-3.4 ppm) due to moisture in the sample overlapped with the signal of 2-Me and 3-Me observed at 3.4-3.5 ppm. In D_2O, the proton signal directly attached to C(2) to which methoxyl groups were attached (labeled as H-2(2-OMe)) appeared as a doublet, and was separated from that attached to C(2) to which did not have methoxyl groups attached (labeled as H-2(2-OH)). In DMSO-d_6 and DMSO-d_6/D_2O, the H-2(2-OH) overlaped with those of H-3 and H-5, and then only H-2(2-OMe) appeared as a triplet signal (B and C in Fig. 2).

According to Nojiri and Kondo[26], the signal of H-2(2-OMe) may shift depending upon conformational changes of the polymer: The conformation may depend on whether 3-OH in either the same or the adjacent anhydroglucose unit is methylated or not. This can account for the separation of H-2(2-OMe) into triplet in DMSO-d_6 and DMSO- d_6/D_2O solvents. In D_2O, H-2(2-OMe) splits into a doublet, probably because

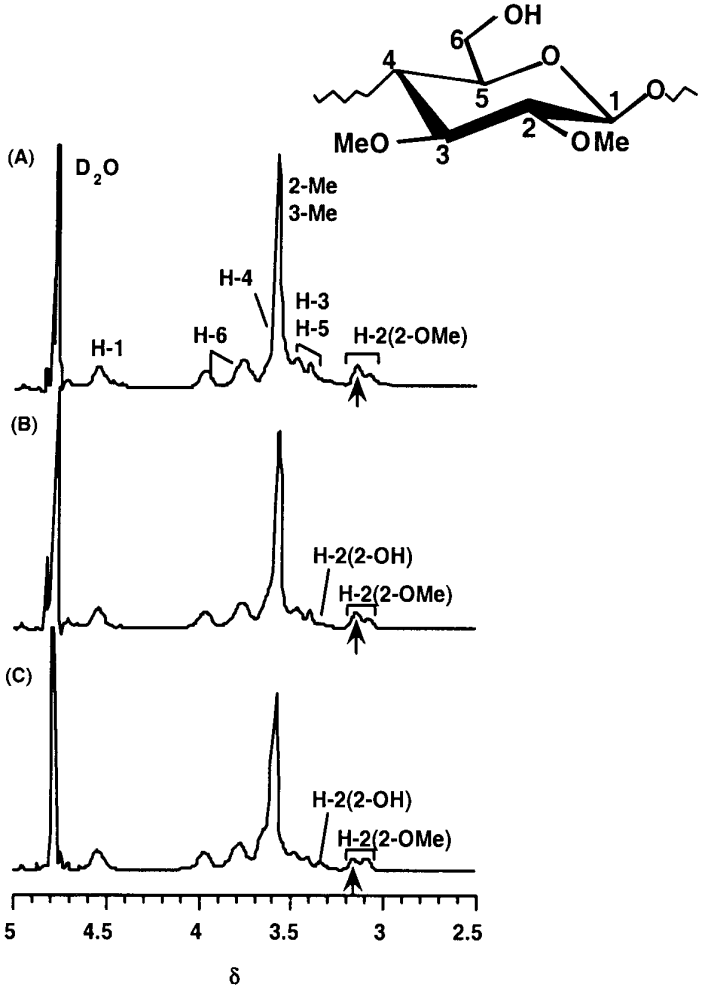


Figure 3 ^{1}H-NMR spectra for a sample series of 2,3-di-*O*-methylcelluloses in D_2O at 15°C: (A) 2,3MC-3, (B) 2,3MC-2, (C) 2,3MC-1.

the methylation of hydroxyl groups at the C(3) position on the adjacent anhydroglucose unit is less influential than in DMSO-d_6 or DMSO-d_6/D_2O solvents. Therefore it would be possible to determine the degree of substitution (DS) at the C(2) position on the basis of the signal intensity at H-2(2-OMe) in D_2O. Furthermore the DS at the C(3) position was calculated by subtracting the DS at the C(2) position from the total DS of the position at the C(2) and the C(3).

The ^{1}H-NMR spectra for samples of 2,3MC series in D_2O at 15 °C are shown in Figure 3. As the series of the samples were systematically and regioselectively methylated in increasing the degree of methylation only at the C(2) and C(3) positions as listed in Table 1, we could follow the change of ^{1}H-NMR signals on the multiple methylation. The relative intensity for left signal of the doublet (see the arrow in Fig.3) for H-2(2-OMe) and the intensity for the signal of 2-Me and 3-Me increased significantly on multiple methylation steps. This indicates that the left signal of the doublet of H-2(2-OMe) is identified as that for H-2 in the anhydroglucose unit where the 3-OH is methylated, and the right signal also identified as that of H-2 with unsubstituted 3-OH on the same unit. Therefore the DS of the individual hydroxyl group at each position can be calculated by the following equations:

$$[H] = (H\text{-}1 + H\text{-}6) / 3 \quad \text{or} \quad [H] = (H\text{-}1) \tag{1}$$

$$DS_{23} = \{(H\text{-}4 + 2\text{-}Me + 3\text{-}Me + H\text{-}5 + H\text{-}3 + H\text{-}2(2\text{-}OMe) + (H\text{-}2(2\text{-}OH)) - (4 \times [H])\} / (3 \times [H]) \tag{2}$$

$$DS_2 = H\text{-}2(2\text{-}OMe) / [H] \tag{3}$$

$$DS_3 = DS_{23} - DS_2 \tag{4}$$

where [H] stands for the intensity of a single proton of each anhydroglucose unit. DS_2 and DS_3 stand for DS at the C(2) and the C(3) positions, respectively, and DS_{23} is the sum of DS_2 and DS_3.

H-2 is the sum of signal areas for H-2(2-OMe) and H-2(2-OH). The first term of the numerator of equation (2) can be determined as the total area of unseparated peaks of H-4, 2-Me, 3-Me, H-5, H-3, and H-2 (shown in Figure 4).

Table I illustrates the DS of the individual methyl group determined by the procedure described above together with the DS determined by gas-chromatography in the parentheses [11]. This table indicates that the total DS increases by the multiple methylation step from 2,3MC-1 to 2,3MC-2, and then to 2,3MC-

H-5 + H-3 + H-2(2-OH), (c) H-2(2-OMe).

3. The DS of the methyl groups on C(2) reaches the saturated value of 1 only at the 2,3MC-3 stage. The signal of substituted methyl groups at the C(6) position in commercial MC (randomly substituted MC) are observed at 3.4 ppm (not shown in these figures). The DS_6 (DS at the 6 position) is calculated as follows:

$$DS_{236} = \{(H\text{-}4 + 2\text{-}Me + 3\text{-}Me + 6\text{-}Me + H\text{-}5 + H\text{-}3 + H\text{-}2) - (4 \times [H])\} / (3 \times [H]) \tag{5}$$

$$DS_6 = \{(6\text{-}Me + H\text{-}5 + H\text{-}3 + H\text{-}2) - (3 \times [H])\} / [H] \tag{6}$$

$$DS_3 = DS_{236} - DS_2 - DS_6 \tag{7}$$

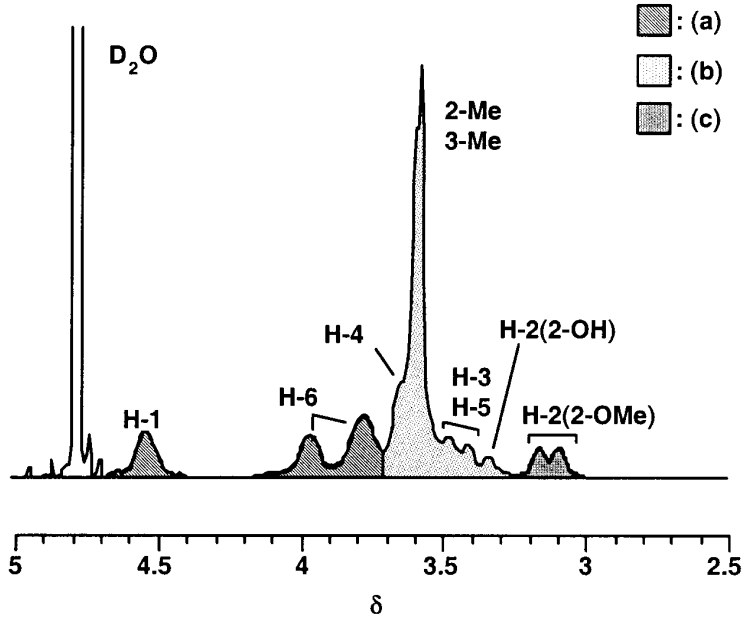


Figure 4 Schematic determination for distribution of the substituent from ^{1}H-NMR spectra of 2,3-di-*O*-methylcelluloses: (a) H-1 + H-6, (b) H-4 + 2-Me + 3-Me +

As the DS values determined using ^{1}H-NMR spectroscopy described in this study agrees in the range of experimental errors (9 %) with those determined by the authentic gas-chromatographic analysis, the present ^{1}H-NMR spectroscopic method in D_2O can be sufficient as a facile and convenient method to measure the distribution of substituents for *O*-MC.

The method to determine the DS of individual positions in methylcelluloses using ^{1}H-NMR thus reported is rapid and reliable and could be employed in cellulose chemistry.

Table I Substituents distribution of *O*-MC samples assigned ^{1}H-NMR method.

Samples	Total DS	DS of methylation at each position		
		2	3	6
2,3MC-1	1.02 (1.33)	0.65 (0.72)	0.38 (0.61)	-
2,3MC-2	1.28 (1.55)	0.77 (0.90)	0.51 (0.65)	-
2,3MC-3	1.85 (1.67)	1.02 (0.99)	0.83 (0.68)	-
Commercial MC	1.71 (1.60)	0.66 (0.69)	0.30 (0.34)	0.75 (0.68)

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