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NMR characterization of cellulose acetate: Chemical shift assignments, substituent effects, and chemical shift additivity

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ABSTRACT

A series of cellulose acetates (CA) with degrees of substitution (DS) ranging from 2.92–0.92 dissolved in dimethylsulfoxide (DMSO)-d_6 and cellulose dissolved in tetrabutylammonium fluoride (TBAF)/DMSO-d_6 were investigated by two-dimensional NMR spectroscopy. The NMR spectroscopic analysis allowed the determination of the ^{1}H and ^{13}C NMR chemical shifts of the eight anhydroglucose units (AGUs) that contain CA: 2,3,6-tri-, 2,3-di-, 2,6-di-, 3,6-di-, 2-mono-, 3-mono-, 6-mono-, and unacetylated AGUs. A comparative analysis of the chemical shift data revealed the substituent effect of acetyl groups at the 2-, 3-, and 6-positions on the ^{1}H and ^{13}C nuclei in the same AGU. In addition, chemical shift additivity could be applied to the ^{1}H and ^{13}C chemical shifts of CA because the chemical shifts of the diacetylated and triacetylated AGUs could be almost completely explained by the acetyl substituent effects at the 2-, 3-, and 6-positions.

1. Introduction

From an analytical point of view, cellulose acetate (CA) is the simplest cellulose ester structure. CA is industrially produced from the esterification of the hydroxyl groups at the 2-, 3-, and 6-positions of the anhydroglucose units (AGUs) of cellulose with acetic anhydride in the presence of acetic acid as a solvent and concentrated sulfuric acid as an esterification catalyst (Malm, Tanghe, & Laird, 1946). The degree of substitution (DS), i.e., the average number of acetyl groups per AGU, ranges from 1.8 to 2.5 in the commercial acetone-soluble products. The properties of CA, such as solubility and viscosity, are closely related to the individual DS at the 2-, 3-, and 6-positions along the cellulose chains, as well as the overall DS (Fox, Li, Xu, & Edgar, 2011; Xu & Edgar, 2012).

In order to determine the CA structure, including the DS and substituent distribution at the monomer level, NMR spectroscopy is applied to get a first look into the individual DS. The CA chains are partially degraded by enzymes. The partial degradation of the CA chains results in a mixture of monomers and oligomers in different molecular ratios. Such mixtures have been separated and characterized in detail using various analytical techniques such as mass spectroscopy (Bashir, Critchley, & Derrick, 2001). Although this approach provides information on the monomer composition as well as the substituent distribution at the oligomeric level, the chemical heterogeneity of CA at the intact polymeric chain level is generally lost. As a new technique for obtaining the structural information of CA at the polymeric chain level, liquid chromatography using multi-step gradient separation has been used and allows the determination of the DS distribution of intact CA chains over a wide DS range of 1.5–2.9. Further, this technique reveals the chemical heterogeneity of the CA structure (Ghareeb & Radke, 2013).

As mentioned above, NMR spectroscopy has been frequently used to reveal the molecular structure of CA, including substituent distribution, DS, etc., because this method provides structural information at the polymer level without any pretreatments. The most well-known method, which was reported by Tezuka and Tsuchiya (1995), is the quantitative analysis of acetyl carbonyl carbons in CA samples, in which unsubstituted hydroxyl groups are perpropionylated by propionic anhydride prior to NMR analysis. The perpropionylated CA shows two sets of three well-resolved carbonyl carbon resonances attributed to the acetyl and propionyl groups substituted at the 2-, 3-, and 6-positions. Thus, the individual DS of CA can be estimated from the relative intensities of the integrals for the three acetyl carbonyl carbons. The perpropionylation of the unsubstituted hydroxyl groups of CA eliminates

Abbreviations: 1D, one-dimensional; 2D, two-dimensional; AGU, anhydroglucose unit; BBO, broadband observe; CA, cellulose acetate; CDCl3, chloroform-d; COSY, correlation spectroscopy; DMAP, *N*,*N*-dimethylaminopyridine; DMSO, dimethylsulfoxide; DS, degree of substitution; HSQC, heteronuclear single quantum coherence; Mn, molecular weight; NMR, nuclear magnetic resonance; TBAF, tetrabutylammonium fluoride; TOCSY, total correlation spectroscopy.

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spectral complications that arise from intra- and intermolecular hydrogen bonds between unsubstituted hydroxyl groups of CA. From the ring carbons in the ^{13}C NMR spectra of CA, acetylation of the hydroxyl group at the 6-position is confirmed by the deshielding of C6, while the C2 and C3 resonances of the AGU overlap the C5 resonance, and therefore do not allow direct observation of the impact of acetyl substitution at the 2- and 3-positions (Goodlett, Dougherty, & Patton, 1971). Instead, acetyl substitution at the 2- and 3-positions of cellulose is thought to result in the shielding of C1 and C4, respectively (Miyamoto, Sato, Shibata, Inagaki, & Tanahashi, 1984), although this has not been directly confirmed by NMR. Therefore, there are still uncertainties about the NMR spectra of CA.

In order to correlate the structure and properties of CA, the chemical shift assignments must be known in detail. In addition, a direct observation of the chemical shift changes that occur with acetyl substitution of cellulose provides useful information about the conformational properties and the molecular dynamics (Kamide & Okajima, 1981). From these points of view, an unambiguous chemical shift assignment for the protons and carbons is required to understand the CA structure. However, the assignment of ring protons and carbons is difficult, with the exception of cellulose triacetate, because the eight AGUs, i.e., 2,3,6-tri-, 2,3-di-, 2,6-di-, 3,6-di-, 2-mono-, 3-mono-, 6-mono-, and unacetylated AGUs, overlap each other in both the ^{1}H and ^{13}C NMR spectra of CA. Moreover, Hikichi, Kakuta, and Katoh (1995) detected nine different ^{1}H—^{1}H spin networks for AGUs in COSY and relayed COSY spectra of CA (DS = 2.46), and suggested that the nine AGUs are due to four different 2,3,6-triacetylated AGUs, two 2,3-diacetylated AGUs, one 2,6-diacetylated AGU, one 3,6-diacetylated AGU, and one 6-monoacetylated AGU based on the ^{1}H chemical shifts (Hikichi et al., 1995). The authors believe that the variation of the ^{1}H—^{1}H spin networks arising from the same AGU is due to different neighbors on either side of the AGU. If this hypothesis is correct, then there are 64 different species possible for each AGU because each AGU can be flanked by any of the seven acetylated AGUs or the unacetylated AGU. Therefore, precise chemical assignment of the AGUs comprising of CA, as well as direct observation of the chemical shift changes accompanying acetylation, still remain a great challenge in the cellulose chemistry.

Recently, the authors reported the two-dimensional NMR characterization of CA with a DS of 2.33, in order to determine the chemical shift assignment of a monomer comprising CA (Kono, 2013a). However, the structural characterization of CAs with a wide DS range is required in order to obtain detailed structural information of CA such as the chemical shift change accompanying a DS change. In this study, CA samples with DS ranging from 2.92 to 0.92 were prepared by the acid-hydrolysis of CA with a DS of 2.92. The precise ^{1}H and ^{13}C chemical shift assignments of the AGUs containing CA were determined by measuring the COSY, TOCSY, and HSQC spectra of the CA samples dissolved in DMSO-d_6 and cellulose powder dissolved in TBAF/DMSO-d_6. DMSO is usually used as an NMR solvent for the characterization of CA in many reports (Qu, Kishimoto, Kishino, Hamada, & Nakajima, 2011; Qu, Kishimoto, Ogita, Hamada, & Nakajima, 2012; Kim & Ralph, 2010; Kim & Ralph, 2014). The TBAF/DMSO solvent system is a powerful solvent that is frequently used for the dissolution of cellulose or wood, and is applied for the homogeneous acetylation of cellulose (Heinze et al., 2000; Heinze, 1998; Köhler & Heinze, 2007; Ass, Frollini, & Heinze, 2004; Lu, & Ralph, 2003). On the basis of the ^{1}H and ^{13}C chemical shift data for each AGU, the substituent effect of the acetyl groups at the 2-, 3-, and 6-positions on the ^{1}H and ^{13}C chemical shifts of each ring proton and carbon were determined. Moreover, the chemical shift additivity observed for CA is discussed with respect to the substituent effects using the chemical shift data obtained in this study.

2. Experimental

2.1. Materials

CA **1** (DS of 2.92 and average molecular weight of 1.8×10^5) and fibrous cellulose powder CF-11 were purchased from Sigma-Aldrich Co. (USA) and Whatman International Ltd. (UK), respectively. All the other chemicals, which were purchased from Kanto Chemicals (Japan) and Wako Pure Chemicals (Japan), were of reagent grade and were used as received.

2.2. Preparation of CA samples

Typically, 1.0 g of CA **1** was dissolved in 20 mL of acetic acid. Concentrated sulfuric acid (0.40 g) was added to the solution followed by water (2.2 mL). The mixture was allowed to react at 358 K for 15, 45, and 80 min to obtain CA **2**–**4**, respectively. At the end of the reaction, a 20% aqueous magnesium acetate solution was added to neutralize the sulfuric acid. The product was precipitated and washed in ethanol. The precipitates were suspended in water, dialyzed using dialysis tubing (Mw 12000 cut-off, Thermo Fisher Scientific Inc., USA) for 3–7 days until completely neutralized, and then freeze-dried. All the samples were stored in a desiccator under vacuum until the time of use.

2.3. Perpropionylation of CA samples

Perpropionylations of CA **1**–**4** were performed according to the previously reported method (Tezuka & Tsuchiya, 1995). Each CA (0.25 g) dissolved in 6 mL of pyridine was allowed to react with 6 mL of propionic anhydride in the presence of 0.1 g of DMAP as a catalyst for 24 h at 358 K. The perpropionylated CA was precipitated and washed with ethanol (100 mL) three times. The sample was reprecipitated from 3 mL of chloroform in 100 mL of ethanol, then filtered and dried at 358 K under vacuum.

2.4. NMR spectroscopy

All the NMR experiments were performed on a Bruker AVIII 500 spectrometer operating at 500.13 MHz for ^{1}H experiments and 125.13 MHz for ^{13}C experiments, and equipped with a 5 mm dual-resonance BBO probe incorporating gradients for the *z*-axis (Bruker BioSpin GmbH, Germany). All the spectra were acquired and processed with Bruker TopSpin version 3.0 on a Windows PC. CA samples (10–20 mg) were dissolved in 600 μL of DMSO-d_6, cellulose samples were dissolved in 600 μL of 5% (w/v) TBAF/DMSO-d_6, and the perpropionylated CA samples (20 mg) were dissolved in 600 μL of $CDCl_3$. Each solution was placed in a 5 mm NMR glass tube (Wilmad-Labglass Co., USA). The sample temperatures were set to 313 K for the samples in $CDCl_3$ and to 363 K for the samples in DMSO-d_6 and TBAF/DMSO-d_6. 1D ^{1}H, 1D ^{13}C, ^{1}H inverse-gated decoupled 1D ^{13}C (Zhou et al., 2007), 2D ^{1}H—^{1}H COSY using a gradient pulse for selection (Trimbre & Bernstein, 1994), 2D ^{1}H—^{1}H TOCSY using the MLEV17 sequence (Bax & Davis, 1985; Braunschweiler & Ernst, 1983) for TOCSY spin-locking, and 2D ^{1}H—^{13}C HSQC via INEPT transfer (Bax, Griffey, & Hawkins, 1983; Bax & Subramanian, 1986) using Echo/Antiecho-TPPI gradient selection (Kay, Keifer, & Saarinen, 1992) with ^{1}H decoupling during acquisition using trim pulses (Bax & Davis, 1985; Braunschweiler & Ernst, 1983) in INEPT transfer were performed for the CA and cellulose samples. 1D ^{1}H, 1D ^{13}C, and ^{1}H inverse-gated decoupled 1D ^{13}C experiments were also performed for the perpropionylated CA samples. In the COSY experiments, a total of 256 t_1 acquisitions with 128 scans per increment were collected. In the TOCSY experiments, the mixing times were 200 ms, and a total of 256 t_1 acquisitions with 128 scans per increment were collected. In the