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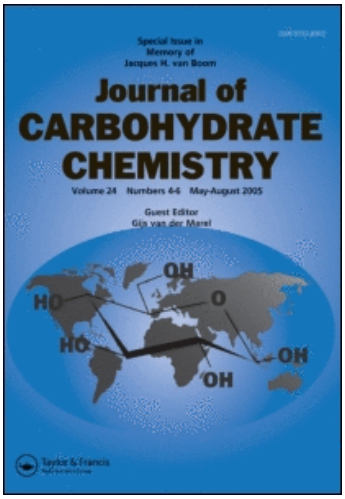

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2D Selective-TOCSY-DQFCOSY Experiment for Identification of Individual Sugar Components in Oligosaccharides

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ABSTRACT

In this paper, we describe an improved 2D selective-TOCSY-COSY experiment for the unambiguous assignment of an individual sugar component in oligosaccharides. We used a DQFCOSY with a pulsed-field gradient instead of a conventional COSY in this improved experiment. The network of proton signals for a selected sugar in an oligosaccharide is observed as the diagonal peaks in the 2D spectrum by use of the first TOCSY period, and the correlation signals between J-coupled neighboring protons are clearly observed as the cross peak, including the signals observed close to the diagonal peaks by the second DQFCOSY development. Even when the signals do not appear in a well-separated form of a 1D spectrum, unambiguous sequential assignment of the proton signals of individual sugar components in an oligosaccharide is achieved by this method.

Key Words: ^{1}H NMR; Selective-TOCSY-DQFCOSY; Unambiguous assignment; Oligosaccharide.

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INTRODUCTION

It is essential to use NMR for the structural analysis of organic compounds in solution. However, the ^{1}H NMR signals of oligosaccharides are observed in a terribly narrow chemical shift range, except for those from the anomeric protons (H-1), making it difficult to assign those proton signals. In order to overcome this problem, some TOCSY-COSY-type experiments[1–3] have been applied to oligosaccharides, and a powerful technique of 2D selective-TOCSY-COSY has been published in review ariticles[4–6] on oligosaccharides. This technique generates a 2D COSY spectrum in combination with the 1D selective-TOCSY spectrum of the desired sugar residue in an oligosaccharide with subsequent development of the second dimension by the COSY pulse sequence. However, the published experiments employ magnitude-calculated COSY with gradient enhancement,[4] which does not give sharp signals, and phase-sensitive COSY without the gradient technique,[5] from which one obtains largely dispersed diagonal peaks. These phenomena make it difficult to assign the proton signals, because some of the cross peaks in a sugar residue are often observed near the diagonal peaks and are hidden by large diagonal peaks on the COSY spectrum. In order to solve this problem in general, DQFCOSY techniques have been developed.[7,8] A DQFCOSY experiment, which can focus on the correlation signal between J-coupled neighboring protons and can purge the spectrum from unwanted coherence, is a well-known technique to improve a COSY spectrum. In addition, this technique can reduce the intensity of such large diagonal signals and make it possible for assignment of correlation signals observed close to the diagonal peaks. Here we describe a 2D selective-TOCSY-DQFCOSY experiment (Figure 1) in order to improve the 2D selective-TOCSY-COSY experiments.[4,5] This experiment contains a concatenated DQFCOSY-block with a pulsed-field gradient to the selective-TOCSY sequence, instead of a conventional COSY-block. In this case, all ^{1}H signals of any selected sugar

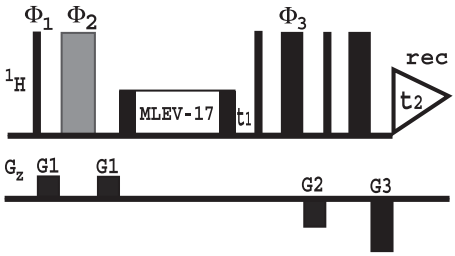

Figure 1. Pulse scheme for the selective-TOCSY-DQFCOSY experiment. Narrow and filled wide rectangular pulses denote 90- and 180-degree flip angles, respectively. A shaded wide rectangular pulse denotes a 180-degree flip angle with shaped pulse. The shape of the 180-degree selective pulse was RE-BURP[9] and the duration was 80 ms. The duration of the repetition time and the isotropic mixing time were set to 2.6 s. and 210 ms, respectively. Quadrature detection was accomplished by incrementing phases of Φ1 and MLEV-17 via the States-TPPI[10] method. The following phase cycling was applied unless x indicates otherwise: Φ 1 = x, −x; Φ 2 = 2x, 2y, 2(−x), 2(−y); Φ 3 = 8x, 8(-x), 8y, 8(−y); receiver = x, −x, −x, x. The sinusoidal gradient pulse length was 1 ms and the ratio was 7.5:−11.5:−23 G/cm for G1:G2:G3.

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component are observed as the diagonal peaks in the 2D spectrum by the selective-TOCSY period, and the correlation signals between J-coupled neighboring protons are observed as the cross peaks by DQFCOSY development.

RESULTS AND DISCUSSION

In order to establish the selective-TOCSY-DQFCOSY experiment, we used a simple disaccharide, lactose. Lactose exits as an anomeric equilibrium mixture, so the ^{1}H NMR spectrum shows three sugar residues, β-D-galactopyranoside, β-D-glucopyranose and α-D-glucopyranose, with its three anomeric signals around 4–5 ppm (Figure 2). As shown in Figure 3A, by measuring the 2D selective-TOCSY-DQFCOSY spectrum in which the anomeric proton of the α-D-glucopyranose unit was selectively excited, the diagonal peaks for α-glucose stand out in bold relief on the 2D spectrum, and the cross peaks evolved by DQFCOSY are shown as neighboring proton signals in the α-D-glucose skeleton. Proton signals from H-1 to H-6 in α-D-glucose were sequentially assigned by connecting the diagonal peak to the cross peak on the spectrum. The assignment of the signals for β-D-glucopyranose and β-D-galactopyranoside units were also obtained by the same method as shown in Figure 3B and Figure 3C, respectively. In addition, in order to improve the intensity of both diagonal peaks and cross peaks corresponding to the signals from H-1 to H-6 in α-D-glucose, the isotropic mixing time in the 1D selective-TOCSY experiment was optimized (Figure 4).

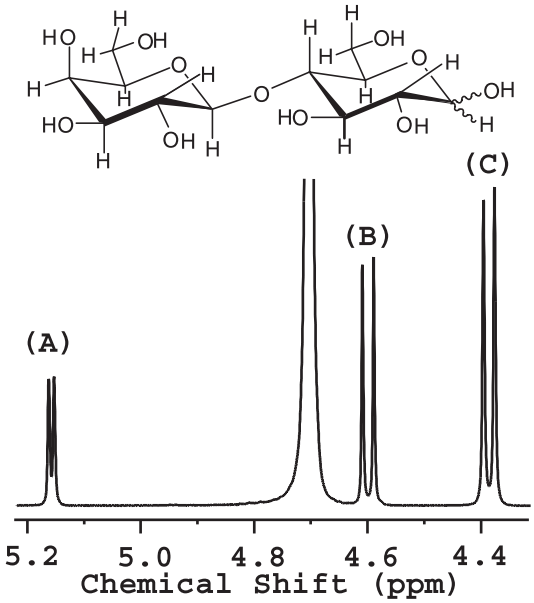

Figure 2. 1D ^{1}H spectrum of anomeric region for lactose in D_2O. Three anomeric proton signals were observed α-D-glucopyranose (A), β-D-glucopyranose (B) and β-D-galactopyranoside (C).

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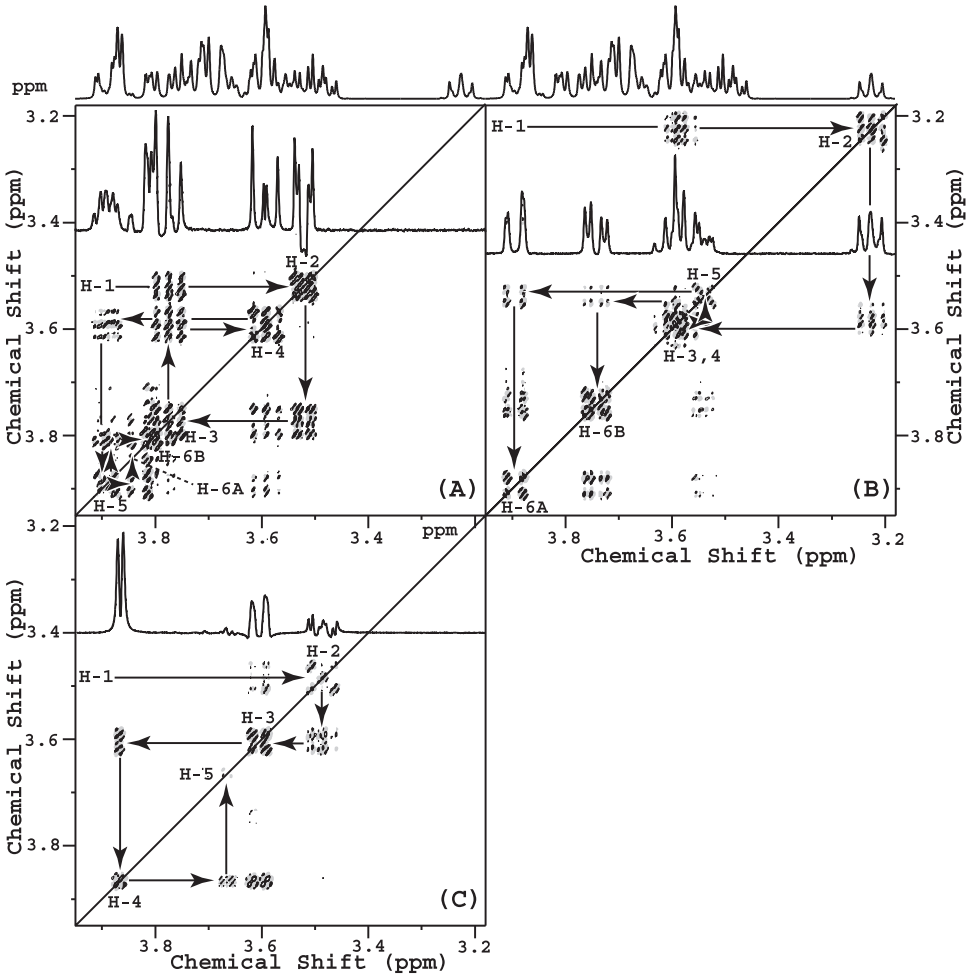

Figure 3. Expanded plots from H-2 to H-6 region in the 2D selective-TOCSY-DQFCOSY spectra of lactose. An anomeric proton signal of α-D-glucopyranose was selectively excited (A). β-D-glucopyranose (B) and β-D-galactopyranoside (C) were selectively excited, respectively. Each of the 1D selective-TOCSY spectra is shown on the 2D spectrum. Normal 1D ^{1}H spectra are shown on top of the 2D spectra.

As shown in Figure 4J, the duration of the isotropic mixing time, 210 ms, was the most suitable for the 1D selective-TOCSY experiment. The same duration was therefore used for this 2D selective-TOCSY-DQFCOSY experiment. As shown in Figure 3, the network of proton signals of a selected sugar unit in the oligosaccharide is observed as the diagonal peaks in the 2D spectrum by use of the first TOCSY period, and the correlation signals between J-coupled neighboring protons are clearly observed as the cross peak, including the signals observed close to the diagonal peaks by the second DQFCOSY development. Even though H-3, H-5, H-6A and H-6B of α-D-glucose are

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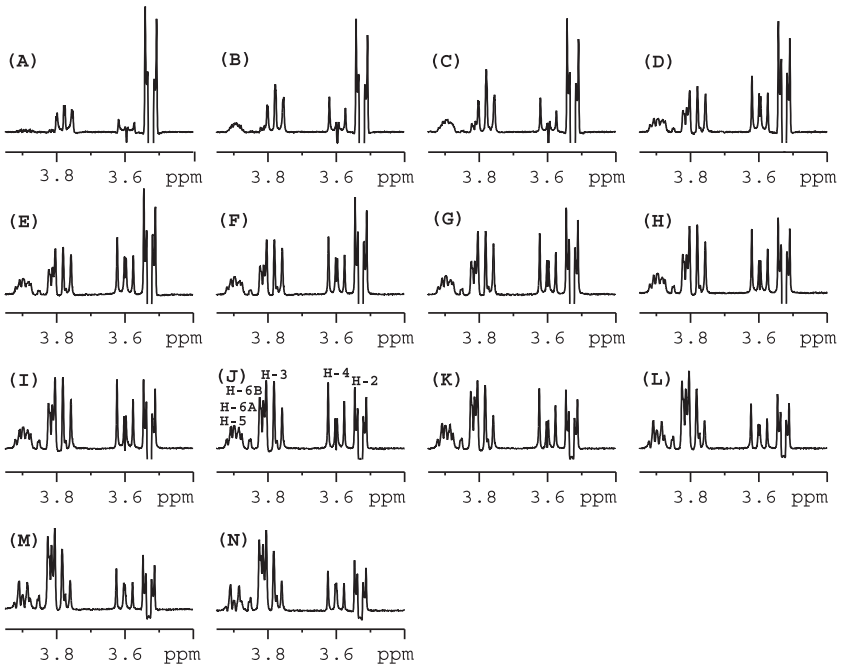

Figure 4. Expanded plots from H-2 to H-6 in 1D selective-TOCSY spectra which selectively excited the anomeric proton signal of α-D-glucopyranose in lactose. The TOCSY isotropic mixing time was changed as follows: 50 ms (A), 75 ms (B), 100 ms (C), 150 ms (D), 160 ms (E), 170 ms (F), 180 ms (G), 190 ms (H), 200 ms (I), 210 ms (J), 220 ms (K), 230 ms (L), 240 ms (M) and 250 ms (N).

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observed in a very narrow region near the diagonal peaks (Figure 3A), unambiguous sequential assignment for proton signals of individual sugar component in the oligosaccharide could be achieved by this method.

In general, D-galactose, *N*-acetylgalactosamine and L-fucose show a very small coupling constant between H-4 and H-5. Therefore, the magnetization transfer from H-4 to H-5 in the above sugars is not efficient in this 2D selective-TOCSY-DQFCOSY method. In Figure 3C, H-5 in β-D-galactopyranoside unit was barely observed as the intensity was not enough. In this case, the 2D-TOCSY-NOESY-TOCSY method, which is an analogue of the 2D-HSQC-TOCSY-NOESY-TOCSY technique,[11] would be a potential technique to observe resonances from H-4 to H-6.

CONCLUSION

An unambiguous sequential assignment of the proton signals of the α and β-D-glucopyranose units in lactose dissolved in D_2O was achieved by a newly established 2D selective-TOCSY-DQFCOSY experiment. We believe that this experiment can be applicable to a larger oligosaccharide as well.

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EXPERIMENTAL

All measurements for lactose (63.7 mmol mL^{-1}) in D_2O were acquired on a Bruker Avance-400 (^1H frequency: 400.13 MHz) spectrometer equipped with an inverse 5-mm TBI ($^1H/^{13}C$/broadband) probe fitted with a Z-gradient coil. The temperature was set to 298 K. The pulse scheme of the 2D selective-TOCSY-DQFCOSY is shown in Figure 1. The data were recorded with a spectral width of 1603 Hz in both dimensions, with 16 scans per FID of 2k data points. Number of t_1 increment was 1k data points. The data were zero-filled to 2k and 2k in both dimensions prior to Fourier transform. A sine-bell window function was applied in both dimensions.

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REFERENCES

1. Inagaki, F.; Shimada, I.; Kohda, D.; Suzuki, A.; Bax, A. Relayed HOHAHA, a useful method for extracting subspectra of individual sugar components of sugar chains. J. Magn. Reson. **1989**, *81*, 186–190.
2. Homans, S.W. Simplification of COSY spectra of oligosaccharides by application of a 2D analog of 3D HOHAHA-COSY. J. Magn. Reson. **1990**, *90*, 557–560.
3. Bircher, H.; Mueller, C.; Bigler, P. Improved sensitivity for the 2D analogue of the 3D HOHAHA-COSY experiment. Magn. Reson. Chem. **1991**, *15*, 726–729.
4. Parella, T. Pulsed field gradients: a new tool for routine NMR. Magn. Reson. Chem. **1998**, *36*, 467–495.
5. Roumestand, C.; Delay, C.; Gavin, J.A.; Canet, D. A practical approach to the implementation of selectivity in homonuclear multidimensional NMR with frequency selective-filtering techniques. Application to the chemical structure elucidation of complex oligosaccharides. Magn. Reson. Chem. **1999**, *37*, 451–478.
6. Duus, J.O.; Gotfredsen, C.H.; Bock, K. Carbohydrate structural determination by NMR spectroscopy: modern methods and limitations. Chem. Rev. **2000**, *100*, 4589–4614.
7. Piantini, U.; Sorensen, O.W.; Ernst, R.R. Multiple quantum filters for elucidating NMR coupling networks. J. Am. Chem. Soc. **1995**, *104*, 6800–6801.
8. Zijl, P.C.M.; Johnson, M.O.; Mori, S.; Hurd, R.E. Magic-angle-gradient double-quantum-filtered COSY. J. Magn. Reson. **1995**, *A113*, 265–270.
9. Geen, H.; Freeman, R. Band-selective radiofrequency Pulses. J. Magn. Reson. **1991**, *93*, 93–141.
10. Marion, D.; Ikura, M.; Tschudin, R.; Bax, A. Rapid recording of 2D NMR spectra without phase cycling. Application to the study of hydrogen exchange in proteins. J. Magn. Reson. **1989**, *85*, 393–399.

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11. Miyazaki, T.; Sato, H.; Sakakibara, T.; Kajihara, Y. An approach to the precise chemoenzymatic synthesis of ^{13}C-labeled sialyloligosaccharide on an intact glycoprotein: a novel one-pot [3-^{13}C]-labeling method for sialic acid analogues by control of the reversible aldolase reaction, enzymatic synthesis of [3-^{13}C]-NeuAc-alpha-(2,3)-Gal-beta-(1,4)-GlcNAc-beta- sequence onto glycoprotein, and its conformational analysis by developed NMR techniques. J. Am. Chem. Soc. **2000**, *122*, 5678–5694.

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