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Sequencing of peracetylated oligosaccharides by rotating-frame nuclear Overhauser enhancement spectroscopy

Janusz Dabrowski*, Andrzej Ejchart*+, Renate Bruntz and Heinz Egge

*Max-Planck-Institut für Medizinische Forschung, Jahnstrasse 29, D-6900 Heidelberg and Institut für Physiologische Chemie, Universität Bonn, Nussallee 11, D-5300 Bonn, FRG

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A combination of two-dimensional total correlation spectroscopy (TOCSY) and rotating-frame nuclear Overhauser enhancement spectroscopy (ROESY) is suggested as the optimum strategy for determining the primary structure of peracetylated oligosaccharides.

Peracetylated oligosaccharide; Oligosaccharide sequencing; NMR, rotating frame ^{1}H-

1. INTRODUCTION

The advantages of peracetylated derivatives vs parent native sugars for structural analysis by ^{1}H-NMR have been described in several papers originating from our laboratories [1–6] and by Gasa et al. [7] and Nishida et al. [8]. The spectra of the former are usually better resolved, the signals more evenly distributed, and the glycosylation sites directly recognizable by the relative ~1 ppm high-field shift for the protons located at these sites, as compared with protons at acetoxylated carbons. On the other hand, sequencing of peracetylated oligosaccharides by inter-residue NOE turned out to be more difficult, due to the much greater mobility of their molecules in chloroform resulting in an NOE close to zero [3], typical of those cases in which $\omega_0\tau_c$, the product of spectrometer angular frequency and molecular correlation time, is approximately equal to unity. To circumvent this difficulty, we have now applied rotating-frame NOE spectroscopy (CAMELSPIN [9], ROESY [10]). With this method, the maximum transient NOE values lie between 38.5% in the extreme narrowing limit, $\omega_0\tau_c \ll 1$, and 67.5% for $\omega_0\tau_c \gg 1$, characteristic of large molecules and/or viscous media, i.e. it does not vanish for any value of $\omega_0\tau_c$. Peracetylated fucopentaose from human milk, Galβ(1→4)[Fucα(1→3)] GlcNAcβ(1→3)Galβ(1→4)Glc-ol, **1**, was taken as a model compound, and its ^{1}H resonances were assigned with the aid of total correlation spectroscopy (TOCSY [11], HOHAHA [12]). The combined application of these two methods led to an unequivocal elucidation of the structure of **1** and we would suggest this combination as an optimum strategy for peracetylated oligosaccharides. Since these are routinely obtained as a step in the purification procedure, structural analysis at this stage should be of some advantage.

Correspondence address: J. Dabrowski, Max-Planck-Institut für Medizinische Forschung, Jahnstrasse 29, D-6900 Heidelberg, FRG

+ *Present address:* Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Abbreviations: HOHAHA, homonuclear Hartmann-Hahn spectroscopy; NOE, nuclear Overhauser enhancement; NOESY, two-dimensional NOE spectroscopy; ROESY, rotating-frame NOE; TOCSY, total correlation spectroscopy

2. EXPERIMENTAL

The preparation of **1** has been described [6]. For ^{1}H-NMR measurements, a 4 mM solution of **1** in $CDCl_3$ containing a

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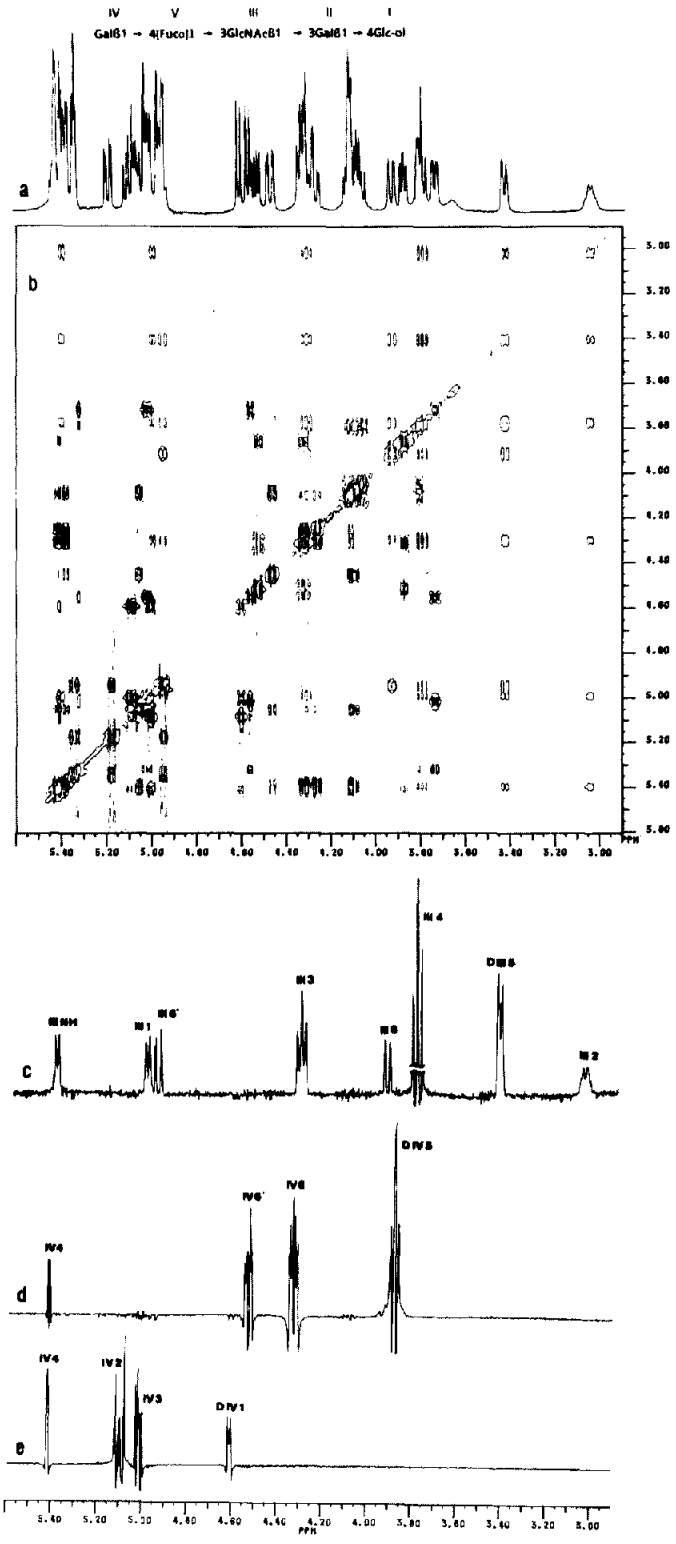
IV V III II I
Galß1 → 4[Fuca]1 → 3GlcNAcß1 → 3Galß1 → 4Glc-ol
a
b
c
d
e
IV4
IV2
IV3
DIV1
IV6'
IV6
DIV6
III4
DIII6
III3
III2
5.40 5.20 5.00 4.80 4.60 4.40 4.20 4.00 3.80 3.60 3.40 3.20 3.00
PPM

trace of Me_4Si was used. All spectra were obtained at a frequency of 500 MHz and a temperature of 303 K on a Bruker AM-500 spectrometer equipped with an Aspect 3000 computer and an array processor. Both of the two-dimensional spectra (TOCSY and ROESY) were measured in a phase-sensitive mode with the use of time-proportional phase increments [13]. The spectral width was 2400 Hz and the spectral size in the time domain was 4096 × 512 (TOCSY), or 2048 × 512 (ROESY). For each t_1, 24 (TOCSY) or 32 (ROESY) transients preceded by 2 dummy scans were accumulated. The sum of the acquisition time and relaxation delay was 3.5 s in both spectra. In the TOCSY spectrum the mixing period of 100 ms for spin locking was applied using the MLEV-17 sequence flanked by two trim pulses of 2.5 ms [14], the locking field strength in frequency units being 7.6 kHz. In the ROESY spectrum [10] the mixing period of 210 ms for spin locking was composed of a train of 8-μs pulses separated by 22-μs delays; an 8 μs pulse corresponded to a 27° flip angle and time-averaged locking field strength was 2.5 kHz. A z-filter was added for suppression of scalar coupling cross-peaks, and the r.f. carrier frequency was offset away from the region of sugar proton resonances during the spin locking time, and then returned to the middle of the spectrum during acquisition for better digital resolution [15]. Both time-domain data were multiplied by a phase-shifted sine-bell window function (phase shifts of $\pi/3$ and $\pi/6$ were used in t_1 and t_2 dimensions, respectively) and zero-filled in both dimensions.

3. RESULTS AND DISCUSSION

The TOCSY spectrum of 1 (fig.1b) contains all the scalar connectivity information on the five closed-spin systems of the five sugar residues. The connectivities between the protons of a given sugar residue are visible in each of its subspectra viewed along the F_2 dimension at the F_1 coordinates of its protons (although the same can be viewed along F_1, the former way is preferable due to a higher digital resolution given for F_2). In principle, each of these subspectra should display cross-peaks for all protons of the sugar residue in question, but due to ineffective magnetization transfer in the case of small coupling constants, some cross-peaks are missing, particularly those depending on the propagation of the magnetization through $^3J_{4,5} \approx 1$ Hz of fucose and the galactoses. Since the whole spectrum is multiply overdetermined, however, this missing information can be complemented quite easily by combining two subspectra for the same sugar residue, in the manner shown in fig.1d,e. The assignments derived from this spectrum are gathered in table 1.

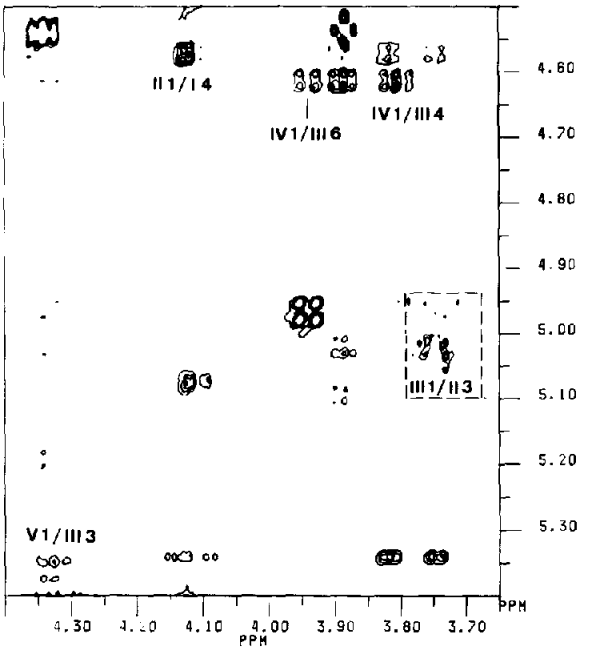

Fig.2. The sequence- and linkage-relevant fragment of the two-dimensional 500 MHz ^{1}H ROESY spectrum. Labelling of protons as in fig.1. Inter-residue NOE cross-peaks defining the sequence are labelled, e.g. V1/III3, denoting Fucα1→3GlcNAc. The III1/II3 cross-peak was weak and had to be reproduced from a lower level of the matrix.

Table 1

Proton chemical shifts for the peracetylated fucopentaose 1 (for numbering see fig.1)

Residue	1	1′(NH)	2	3	4	5	6	6′
I	4.29	4.33[a]	5.44	5.41	4.14	5.08	4.12	4.48
II	4.58		5.04	3.76	5.34	3.83	4.09	4.14
III	5.00	5.43[b]	3.05	4.33	3.82	3.46	3.95	4.97
IV	4.62		5.11	5.03	5.44	3.90	4.34	4.55
V	5.35		4.95	5.20	5.37	4.93	1.21	–

[a] H-1′
[b] N-H

Fig.1. The sugar proton region of 500 MHz ^{1}H-NMR spectra of the peracetylated fucopentaose 1 (acetyl groups are not indicated in the formula). (a) A conventional one-dimensional spectrum. (b) Two-dimensional TOCSY spectrum. (c–e) Cross-sections (subspectra) through the diagonal cross-peaks (labelled with D) of the two-dimensional TOCSY matrix. Arabic numbers denote protons of the sugar residues labelled with Roman numerals.

Although the high-field-shifted resonances of aglyconic protons located at glycosidic bridges clearly indicate the glycosylation sites, it is not possible to determine the sequence from these shifts. On the other hand, the ROESY spectrum exhibits all of the dipolar connectivities required for sequence determination. Thus, along with intra-residue NOEs for synaxial and equatorial/axial protons, which confirmed the assignments derived from the TOCSY spectrum, the inter-residue NOEs for the anomeric and transglycosidic protons defining the four segmental sequences are also apparent (fig.2). Concerning the Gal-IV/GlcNAc-III disaccharide segment, the simultaneous occurrence of two strong inter-residue cross-peaks (IV1/III4 and IV1/III6) could be misleading; this uncertainty is fictional, however, since the glycosylation site at C-4 is unambiguously determined by the high-field shift of the H4 resonance of Glc-III. Thus, the IV1/III6 cross-peak points to a particular conformation concerning the IV/III glycosidic linkage and the CH_2OAc group of the Glc-III residue. Since the interpretation of transglycosidic NOEs for the remaining V1/III3, III1/II4 and II1/I4 linkages is unequivocal, the whole primary structure of **1** is unambiguously determined by the combination of a TOCSY and a ROESY spectrum.

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REFERENCES

[1] Dabrowski, U., Egge, H. and Dabrowski, J. (1983) Arch. Biochem. Biophys. 224, 254–260.
[2] Dabrowski, J., Egge, H. and Dabrowski, U. (1983) Carbohydr. Res. 114, 1–9.
[3] Dabrowski, J., Dabrowski, U., Hanfland, P., Kordowicz, M. and Hull, W.E. (1986) Magn. Reson. Chem. 24, 59–69.
[4] Dabrowski, J., Ejchart, A., Kordowicz, M. and Hanfland, P. (1986) Magn. Reson. Chem. 25, 338–346.
[5] Dabrowski, J. (1987) in: Methods in Stereochemical Analysis (Croasmun, W.R. and Carlson, R.M.K. eds) vol.9, pp.349–386, VCH.
[6] Bruntz, R., Dabrowski, U., Dabrowski, J., Ebersold, A., Peter-Katalinic, J. and Egge, H. (1988) Biol. Chem. Hoppe-Seyler 369, 257–273.
[7] Gasa, S., Nakamura, M., Makita, A., Ikura, M. and Hikichi, K. (1986) Eur. J. Biochem. 155, 603–611.
[8] Nishida, T., Enzell, C.R. and Morris, G.A. (1986) Magn. Reson. Chem. 24, 179–182.
[9] Bothner-By, A.A., Stephens, R.L., Lee, J., Warren, C.D. and Jeanloz, R.W. (1984) J. Am. Chem. Soc. 106, 811–813.
[10] Bax, A. and Davis, D.G. (1985) J. Magn. Reson. 63, 207–213.
[11] Braunschweiler, L. and Ernst, R.R. (1983) J. Magn. Reson. 53, 521–528.
[12] Davis, D.G. and Bax, A. (1985) J. Am. Chem. Soc. 107, 2820–2821.
[13] Marion, D. and Wüthrich, K. (1983) Biochem. Biophys. Res. Commun. 113, 967–974.
[14] Bax, A. and Davis, D.G. (1985) J. Magn. Reson. 65, 355–360.
[15] Rance, M. (1987) J. Magn. Reson. 74, 557–564.