Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Magnetic Resonance 170 (2004) 156–163
JMR Journal of Magnetic Resonance
www.elsevier.com/locate/jmr

Rapid 3D NMR using the filter diagonalization method: application to oligosaccharides derivatized with ^{13}C-labeled acetyl groups

Geoffrey S. Armstrong,[a,1] Kristin E. Cano,[a] Vladimir A. Mandelshtam,[a] A.J. Shaka,[a] and Brad Bendiak[b,*]

[a] *Chemistry Department, University of California, Irvine, CA 92697-2025, USA*
[b] *Department of Cell and Developmental Biology and Biomolecular Structure Program, University of Colorado Health Sciences Center, Box B111, 4200 East Ninth Avenue, Denver, CO 80262, USA*

Received 7 April 2004; revised 4 June 2004
Available online 20 July 2004

Abstract

Rapid 3D NMR spectroscopy of oligosaccharides having isotopically labeled acetyl "isotags" was made possible with high resolution in the indirect dimensions using the filter diagonalization method (FDM). A pulse sequence was designed for the optimal correlation of acetyl methyl protons, methyl carbons, and carbonyl carbons. The multi-dimensional nature of the FDM, coupled with the advantages of constant-time evolution periods, resulted in marked improvements over Fourier transform (FT) and mirror-image linear prediction (MI-LP) processing methods. The three methods were directly compared using identical data sets. A highly resolved 3D spectrum was achieved with the FDM using a very short experimental time (28 min).

Keywords: Filter diagonalization method; Multi-dimensional NMR; Constant-time; C-13 labeling; O-acetylation; Oligosaccharides

1. Introduction

In the quest to provide detailed structural and dynamic information about macromolecules of ever-larger size and complexity, the greatest single limitation in NMR spectroscopy is the overlap of cross peaks. At some upper size limit, the assignment of many signals and deduction of their associated spin systems simply becomes impossible. Procedures for diminishing cross peak area or volume, such as decoupling in indirect or direct dimensions, tROSY [1], or extracting "singlet" spectra using appropriate projections of specific experiments [2] do improve the situation significantly, but by far the most potent improvement in spectral dispersion ensues by extending experiments to additional dimensions. Such extensions do engender issues of concern. These include time limitations when increasing the number of increments in indirect dimensions, lower resultant resolution in indirect dimensions using standard processing methods, and diminishing returns in signal intensity during multiple concatenated magnetization transfers and indirect detection periods.

The filter diagonalization method (FDM) [3–7] has emerged as a powerful tool for the analysis of higher dimensionality experiments. In contrast to the Fourier transform (FT), which must be applied sequentially to orthogonal dimensions, the FDM is a true multi-dimensional method. The FDM is able to process the complete data set, taking advantage of the large signal "area" present in multi-dimensional experiments. However, not all pulse sequences are appropriate for the FDM. Therefore, the conjoint design of new pulse sequences with FDM processing as a built-in goal must be considered for optimizing its potential. In particular, the

* Corresponding author. Fax: 1-303-315-4729.
E-mail address: Brad.Bendiak@UCHSC.edu (B. Bendiak).
[1] Present address: Department of Cell and Developmental Biology, University of Colorado Health Sciences Center, Box B111, 4200 East Ninth Avenue, Denver, CO 80262, USA.

1090-7807/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmr.2004.06.002

FDM has been used in the analysis of a 3D HNCO experiment that was obtained with only a few increments in both indirect dimensions [8]. The successful application of the FDM to proteins is just one illustration of its fundamental advantages that may be brought to bear on any group of molecules with aptly arranged nuclei.

Investigating the role of complex carbohydrates in cellular function is a rapidly growing field of study. It is now evident that this class of molecules is responsible for numerous types of intercellular recognition processes [9]. Disruption of these processes can have far reaching consequences, and result in various hereditary afflictions and oncologic events [10]. Unfortunately, elucidation of the structure of oligosaccharides is complicated by the need to define the stereochemistry of very closely related monosaccharide units, and the plethora of configurations that arise from different linkages between these units. From the perspective of NMR spectroscopy, they are complicated by a very crowded proton spectrum even for small carbohydrates and isotopically labeled oligosaccharides are usually not available. Furthermore, the issues that confound structural assignment of carbohydrates cannot draw on pulse sequences employed for resolving protein structure. New NMR methods are necessary, tailored to the unique features of these molecules.

A method to alleviate these problems has been described previously [11,12]. It involves the peracetylation of oligosaccharides with doubly ^{13}C-labeled acetyl groups (one of a group of derivatives that we have termed "isotags"). This provides several advantages to structural elucidation of carbohydrates, but the most important are first, that the spectral range of the sugar ring protons is increased almost three-fold. Second, the ^{13}C-labeled acetyl groups afford a point of entry for magnetization to be transferred into the saccharide units, allowing for analysis of the coupling networks through multi-dimensional correlations to acetyl proton and carbon frequencies. Third, the through-bond coupling of the acetyl carbonyl group to the sugar ring proton at the site of acetylation identifies all positions that were formerly free hydroxyl groups. By inference, this enables *the site of a glycosidic linkage* to be determined unambiguously, as positions of acetylation and glycosylation are mutually exclusive. However, as with other macromolecules, the upper limit on the number of spin systems that may be simultaneously elucidated is primarily restricted by the overlap of crosspeaks.

Previously, the correlation of the acetyl proton and carbon frequencies to sugar ring proton frequencies was carried out with the H(CMe)COH-HEHAHA or (HCMe)COH-RELAY experiments [11,12]. They permitted the sugar ring proton frequencies to be correlated with up to three independent frequencies of the acetyl groups (CO, CMe, and HMe). However, experiments were performed by skipping one acetyl frequency in combinations of 3D experiments that used conventional (FT) processing methods. Clearly it would be propitious to apply the FDM to extend these analyses to higher dimensional experiments and still retain sufficient resolution in the indirect dimensions. In this paper, we report a 3D experiment designed specifically for processing using FDM [6,8]. The goal was to transfer magnetization from HMe to CMe and then to CO with an evolution time at each step, resulting in a 3D experiment that prepared magnetization for transfer into the sugar rings. This experiment was performed using relatively few increments in the indirect dimensions and a correspondingly short experimental time, paving the way for rapid 4D experiments designed around FDM processing. In addition, we experimentally demonstrate how well the FDM, as compared with FT and MI-LP, estimates frequencies of signals of this type in which redundant regions having narrower natural linewidths than proteins can be discerned with high resolution in the indirect dimensions.

2. Theory

A derivation of the multi-dimensional FDM has been discussed previously [4] and as such will not be dealt with in detail here. However, a brief introduction to the development of the method in one-dimension is useful. The FDM is based on a form of the auto-regression assumption, relating the signal, $c(n\tau)$, to the evolution, under the operator $\hat{U}$, of the initial state $|\Phi)$:

$$c(n\tau) := c(n) = (\Phi|\hat{U}^n|\Phi), \ (n = 0, \ldots, N-1). \tag{1}$$

This can be shown to be equivalent to assuming that the signal ($c(n)$) is composed of a sum of complex exponentials with amplitudes, phase, frequencies and decay constants. These parameters can be obtained by solving for the eigenvalues (u_k) and eigenvectors ($(\Upsilon_k|$) of the operator $\hat{U}$.

$$c(n) = \sum_{k=1}^{K} d_k \mathrm{e}^{-\mathrm{i}n\omega_k\tau} = \sum_{k=1}^{K} (\Phi|\Upsilon_k) u_k^n (\Upsilon_k|\Phi). \tag{2}$$

Here, the round brackets denote the complex–symmetric nature of the operator ($(a|b) = (b|a)$). An appropriate basis for the solution of this problem is the Fourier basis with a uniformly chosen frequency grid (φ_j) of size K_{win}:

$$|\Psi_j) = \sum_{n=0}^{N/2-1} \mathrm{e}^{-\mathrm{i}n\tau\varphi_j} |\Phi_n) \ , \tag{3}$$

where $\{|\Phi_n), \ (n = 0, \ldots, N/2-1)\}$ is defined by $(\Phi_n|\Phi_m) = c(n+m)$. This basis localizes the matrix elements of $\hat{U}$ close to the diagonal, allowing the calculation to be split into "windows" of size $K_{\mathrm{win}} \times K_{\mathrm{win}}$, improving the speed of the diagonalization step. The basis allows the solution of the generalized eigenvalue problem:

$$\mathbf{U}_1\mathbf{B}_k = u_k\mathbf{U}_0\mathbf{B}_k. \tag{4}$$

The matrix $\mathbf{U}_1$ is the representation of $\hat{U}$, and the matrix $\mathbf{U}_0$ accounts for the non-orthogonal nature of the Fourier basis. The parameters ($d_k = \sqrt{\mathbf{B}_k^{\mathrm{T}}\mathbf{U}_0\mathbf{B}_k}$ and $\omega_k = \mathrm{i}\ln(u_k)/\tau$) can now be obtained, and the spectrum generated using an appropriate function (i.e., gaussian or Lorentzian lineshape).

Eq. (4) is the FDM in its one-dimensional form, but it can be easily generalized to multiple dimensions [4,7]. For 3D FDM [8], this amounts to the solution of three separate generalized eigenvalue problems:

$$\begin{aligned}\mathbf{U}_1\mathbf{B}_k &= u_{1k}\mathbf{U}_0\mathbf{B}_k,\\ \mathbf{U}_2\mathbf{B}_k &= u_{2k}\mathbf{U}_0\mathbf{B}_k,\\ \mathbf{U}_3\mathbf{B}_k &= u_{3k}\mathbf{U}_0\mathbf{B}_k.\end{aligned} \tag{5}$$

While on the surface this appears to be a trivial problem, the matrices $\mathbf{U}_0$, $\mathbf{U}_1$, $\mathbf{U}_2$, and $\mathbf{U}_3$ are quite complicated in that they each contain information from all three dimensions. In other words, they are built up from the 3D signal, $c(n_1, n_2, n_3)$. Each eigenvalue problem, therefore, has available to it $N_1 \times N_2 \times N_3$ data points. While the indirect dimensions may have very few points (N_2, $N_3 \ll N_1$), their product may still be very large. This allows the extraction of numerous parameters from relatively small (by Fourier transform standards) data sets. This is a direct result of the multi-dimensional nature of the FDM.

While the large multi-dimensional data set results in a much higher resolving power for the FDM, it complicates the eigenvalue problem with a large number of solutions that may be very close to zero. This problem is referred to as ill-conditioning, and it may result in narrow, intense artifacts in the resulting spectrum. For this reason, the solution of the generalized eigenvalue problem must be regularized to exclude solutions that are not sufficiently "smooth." This has been employed previously in the algorithm FDM2k [5]. The regularization is employed by recasting the generalized eigenvalue problem into a slightly different form:

$$\begin{aligned}\mathbf{U}_0^{\dagger}\mathbf{U}_1\mathbf{B}_k &= u_{1k}\{\mathbf{U}_0^{\dagger}\mathbf{U}_0 + q^2\}\mathbf{B}_k,\\ \mathbf{U}_0^{\dagger}\mathbf{U}_2\mathbf{B}_k &= u_{2k}\{\mathbf{U}_0^{\dagger}\mathbf{U}_0 + q^2\}\mathbf{B}_k,\\ \mathbf{U}_0^{\dagger}\mathbf{U}_3\mathbf{B}_k &= u_{3k}\{\mathbf{U}_0^{\dagger}\mathbf{U}_0 + q^2\}\mathbf{B}_k.\end{aligned} \tag{6}$$

By changing the value of the regularization parameter (q), one can remove the ill-conditioning and obtain a stable spectral estimate.

The FDM can take advantage of particular properties of certain NMR experiments. Constant-time (CT) experiments [13], for example, are particularly suited to the FDM due to the lack of decay of the signal in the indirect dimensions. This results in purely Lorentzian behavior of the signal, which fit the assumption of the FDM, and a constant relative contribution of noise throughout the increments. As the FDM requires fewer data points, shorter CT periods can be processed, resulting in greater signal-to-noise throughout the signal. This also becomes relevant if T_2 relaxation is faster for specific nuclei. In addition to this, the FDM can process both phase-sensitive data sets of a CT experiment as one large data set, effectively doubling the amount of data available to the method [6]. For a 3D experiment with two CT dimensions, the FDM uses four times the amount of information, quadrupling the resolving power of the method [8]. For these reasons, the resolving capability of the FDM using a CT type signal is much greater, allowing the data to be collected in a much shorter time. However, numerical imperfections arising from the zero-decay signal can cause artifacts in the resulting spectral estimate produced by the FDM. It is therefore important to employ a slight smoothing of the peaks when the spectrum is generated. This smoothing parameter (Γ) is usually on the order of the natural linewidth of the peaks.

3. Experimental

Nigeran tetrasaccharide (α-D-Glc-[1 → 3]-α-D-Glc-[1 → 4]-α-D-Glc-[1 → 3]-α-D-Glc) (Sigma N7263) was chosen as a challenging complex carbohydrate due to the similarity of the chemical shifts for each monosaccharide. It was reduced using sodium borohydride and peracetylated with uniformly labeled acetic anhydride in the same manner as previously reported [12,18]. The sample (2 mM) was dissolved in $CDCl_3$ with a layer of D_2O above [12]. A 3D CT experiment was designed (Fig. 1) to transfer magnetization from the methyl protons (HMe) of the labeled acetyl group, to the methyl carbons (CMe), and to the carbonyl carbons (CO), then back again for direct detection. CT evolution periods were employed for both the indirect dimensions. In addition, shaped adjustable, broadband, sech/tanh-rotation uniform selective excitation (ABSTRUSE) pulses and broadband inversion pulses (BIP) [14,15] were used to improve overall magnetization transfer. The experiments were performed at 25 °C on a Varian UNITY-INOVA 500 MHz spectrometer equipped with a triple resonance gradient probe. The phase sensitive data was acquired using States-TPPI in both indirect dimensions [16]. The delays (Δ_1, Δ_2, and Δ_3) were chosen to optimize the transfer of magnetization through the sequence. The first two delays (Δ_1 and Δ_2) correspond to $1/(4J_{\mathrm{CH}})$ and $1/(4J_{\mathrm{CC}})$, respectively to optimize the INEPT transfer from proton to carbon and from carbon to carbon. While this choice for Δ_2 only allows a few increments in the methyl dimension, this is not a drawback for the FDM. The final delay ($\Delta_3 = 3/(2J_{\mathrm{CC}})$) was chosen to allow the maximum amount of magnetization to be transferred, while maintaining a suitable number of in-

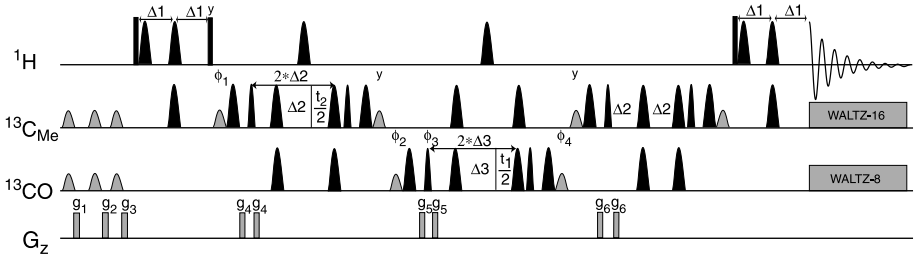

Fig. 1. Pulse sequence for the 3D constant-time $HC_{Me}CO$ experiment. In this diagram, narrow bars are used to represent hard 90° pulses and shaped pulses are indicated by black and grey curves for 180°, and 90° pulses, respectively. The series of three shaped pulses (one 90° pulse of duration 2.0 ms and two 180° pulses with durations 2.0 and 1.0 ms, respectively, separated by a 1.0 ms delay) represent ABSTRUSE [14] sequences of hyperbolic secant pulses. It should also be noted that the time for this pulse need not be subtracted from the evolution time so long as an exact reverse sequence is used after the period. All other 180° shaped pulses are BIP [15] pulses, optimized for the decoupler or transmitter offset. The proton BIPs are all 40 μs long, and the carbon BIPs are 60 μs for the proton INEPT and 140 μs for the broadband inversion of both methyl and carbonyl. The sequence begins with an INEPT transfer from the protons to the methyl carbons ($\Delta_1 = 1.9$ ms) followed by an ABSTRUSE sequence applied at the C_{Me} frequency (−9243 Hz), and constant-time evolution on the methyl carbon ($\Delta_2 = 4.2$ ms). After the evolution, an ABSTRUSE at the CO frequency (+9584 Hz) starts the constant-time evolution on CO ($\Delta_3 = 25$ ms). The magnetization is moved back to the methyl protons with two reverse INEPT type sequences. WALTZ decoupling is applied on the carbon channel during acquisition. The decoupling was modulated to apply 1.375 kHz to the methyl frequency and 0.687 kHz to the CO frequency. For this reason on the diagram it has been represented as separate WALTZ-16 and WALTZ-8 sequences on each frequency. Matched gradients have also been applied during each of the ABSTRUSE excite pulses to select only the desired magnetization. The durations and strengths of the gradients are $g_1 = (0.5\text{ ms}, 2.3\text{ G/cm})$, $g_2 = (0.7\text{ ms}, 2.3\text{ G/cm})$, $g_3 = (0.86\text{ ms}, 2.3\text{ G/cm})$, $g_4 = (0.2\text{ ms}, 2.3\text{ G/cm})$, $g_5 = (0.2\text{ ms}, 3.8\text{ G/cm})$, and $g_6 = (0.2\text{ ms}, 5.4\text{ G/cm})$. The phase cycling was $\phi_1 = 4(x)$, $\phi_2 = 4(x)$, $\phi_3 = (x, y, x, y)$, $\phi_4 = (x, x, -x, -x)$, and $\text{rec} = (x, -x, -x, x)$. The collection of phase-sensitive data was accomplished using States-TPPI [16] incrementation of ϕ_1 for the methyl carbon dimension and ϕ_2 for the carbonyl carbon dimension. In addition, the phase of all preceding carbon pulses was incremented [17].

crements during the evolution time. The 3D data was obtained with only four increments in the methyl dimension, 16 increments in the carbonyl dimension and 512 points of acquisition. Using four transients, this acquisition took only 28 min. In addition, two 2D experiments were conducted with longer constant-time intervals (and more increments) in each of the indirect dimensions for comparison. FDM calculations were performed on a dual-CPU 2.0 GHz Apple PowerMac G5 with 2 Gb RAM. The FT and mirror-image linear prediction (MI-LP) results were obtained using NMRPipe [19].

4. Results and discussion

The 3D experiment was tailored to oligosaccharides derivatized with doubly ^{13}C-labeled acetyl groups to conjointly exploit the advantages offered both by isotags and by processing with the FDM. Results comparing different processing methods, and related 2D experiments having more increments in the indirect dimension are shown in Figs. 2 and 3. It is clear that by processing the 3D data coherently, taking full advantage of its multi-dimensionality, the FDM is able to produce a high-resolution 3D spectrum that far outstrips conventional methods of processing. Using exactly the same data set, the direct comparison between the FDM and FT processing is illustrated in panels Figs. 2A and D, and in panels Figs. 3A and D. Similarly, a direct comparison between the FDM and MI-LP processing is shown in panels Figs. 2A and B, and panels Figs. 3A and B. What becomes conspicuously evident in these comparisons is the basic essence of the FDM: multidimensional experiments do not require numerous increments in all indirect dimensions for high resolution. Rather, as long as the product of the increments (signal "area") is large enough, the entire spectrum can be resolved in every dimension. In addition, note that 2D experiments processed by conventional methods require many more increments in the indirect dimension to approach the degree of resolution furnished by the same projections of the 3D experiment processed by the FDM (compare panels Figs. 2A and C, 3A and C).

Regarding the FDM processing, a single window of size $200 \times 250 \times 250$ Hz was processed, using $400 \times 4 \times 16$ points in the proton, methyl carbon and carbonyl carbon dimensions, respectively ($K_{\text{win}} = 344$). This calculation took only 25 s to complete. A range of regularization and smoothing parameters was tested to ensure that the optimal result was obtained. It was found that the result was very stable over a large range of regularization parameters (value of q in Eq. (6), from 0.001 to 0.05), and required only a small smoothing ($\Gamma = 1$ Hz in each dimension).

These experiments also illustrate some of the difficulties associated with carbohydrate NMR. As the individual sugar units are very similar to each other, the chemical shifts of nuclei within acetyl groups have a limited spectral range. In this compound, for example,

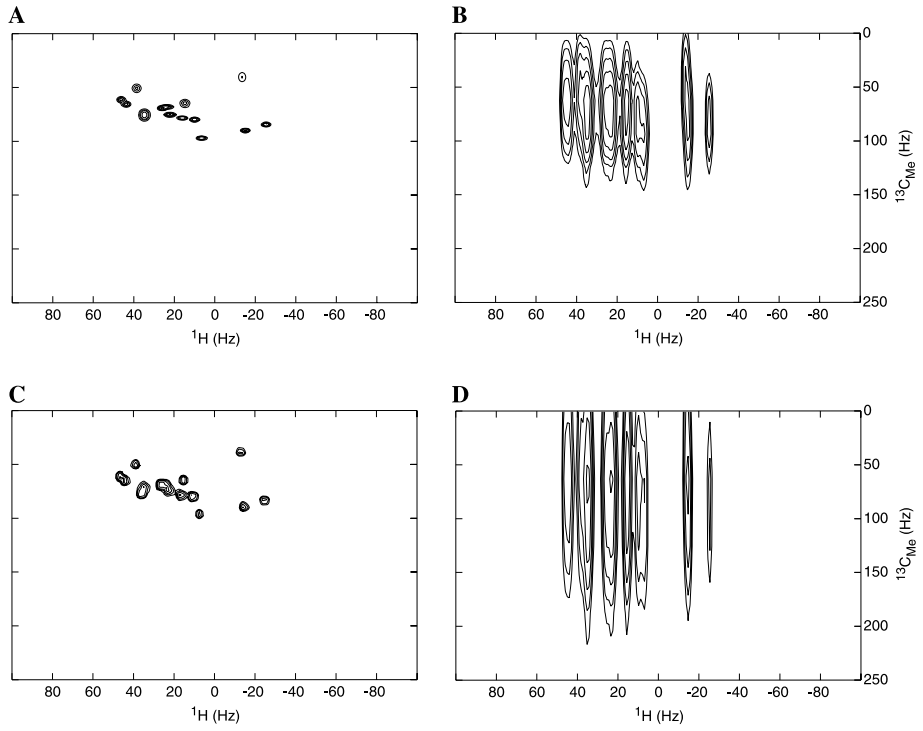

Fig. 2. Spectra that compare different processing methods for obtaining HMe, CMe, and CO correlations among nuclei of acetyl groups of the peracetylated nigeran oligosaccharide-alditol. Using the identical 3D data set ($512 \times 4 \times 16$ points), panels A, B, and D compare the resolution obtained after processing with the FDM (A), MI-LP (B) and FT (D), showing the HMe–CMe projection. For comparison, a higher resolution FT spectrum from a longer (512×62 points, $\Delta_3 = 62.5$ ms) 2D experiment is shown in panel C. The FDM calculation was performed in a single window using $400 \times 4 \times 16$ points in the proton, methyl and carbonyl dimensions, respectively. A regularization of $q = 0.005$ was applied with a smoothing of $\Gamma = 1$ Hz in each dimension. The results from processing the 3D experiment using the FDM are in agreement with the longer 2D experiment processed with FT (C). Note that the widths in the longer 2D experiment (C) are artificially increased by the truncation of the data, and are therefore slightly larger than the widths estimated by the FDM. Much higher resolution was obtained in the indirect dimension of the 3D experiment by processing with the FDM (A) as compared to either the MI-LP (B) or FT (D). With only four points in the indirect dimension of this FDM calculation (A) the resolution obtained is comparable to the FT calculation with almost 16 times the number of increments (C).

the methyl carbons only occupy a range of about 100 Hz (less than a ppm). The carbonyl carbons improve the resolving power somewhat, but despite the addition of this dimension there are still two peaks that overlap in all dimensions for this compound (34 Hz in HMe, 73 Hz in CMe, and 47 Hz in CO). Therefore, while there should be 15 peaks in each spectrum (due to each of the 15 acetyl groups) only 14 can be discerned. Although the FDM calculation shows marked resolution improvement, particularly in the indirect dimensions, over that obtained through the application of MI-LP, there is only so much spectral dispersion that 3D can provide. The probability that three frequencies are nearly coincidental is not high, yet does point out the need to extend correlations to higher dimensions.

Apart from the resolution obtained in each of the 2D projections of the 3D spectrum, the FDM also improves the result when viewing individual planes. Fig. 4 shows the result from two adjacent "slices" of the 3D spectrum. The FDM has complete resolution of the peaks in both spectra and shows no overlap between the planes. The MI-LP, despite being applied in both dimensions, cannot offer the same resolution as that of the FDM, and all peaks from the two separate planes show up in both spectra. It should also be noted that while the FDM planes were constructed for direct comparison with the MI-LP, any frequency range can be produced using the FDM. Therefore, the resolution (thickness) of the plane is not restricted by the number of increments in that dimension. This significantly simplifies the process of assigning frequencies in a 3D data set. Moreover, we have shown experimentally that the FDM is capable of providing high-resolution frequency estimates of signals in the indirect dimensions having redundancy over smaller spectral precincts with narrower natural linewidths than proteins, a typical scenario encountered with these isotag-derivatized oligosaccharides.

While the FDM was applied in this case to a sample that has only 15 sets of resonances, it is easy to visualize the application to larger systems. For instance, oligosaccharides that have more than four sugar units will give more complicated spectra, which will be even more difficult to resolve using the FT. In addition, mixtures of small peracetylated oligosaccharides will lead to

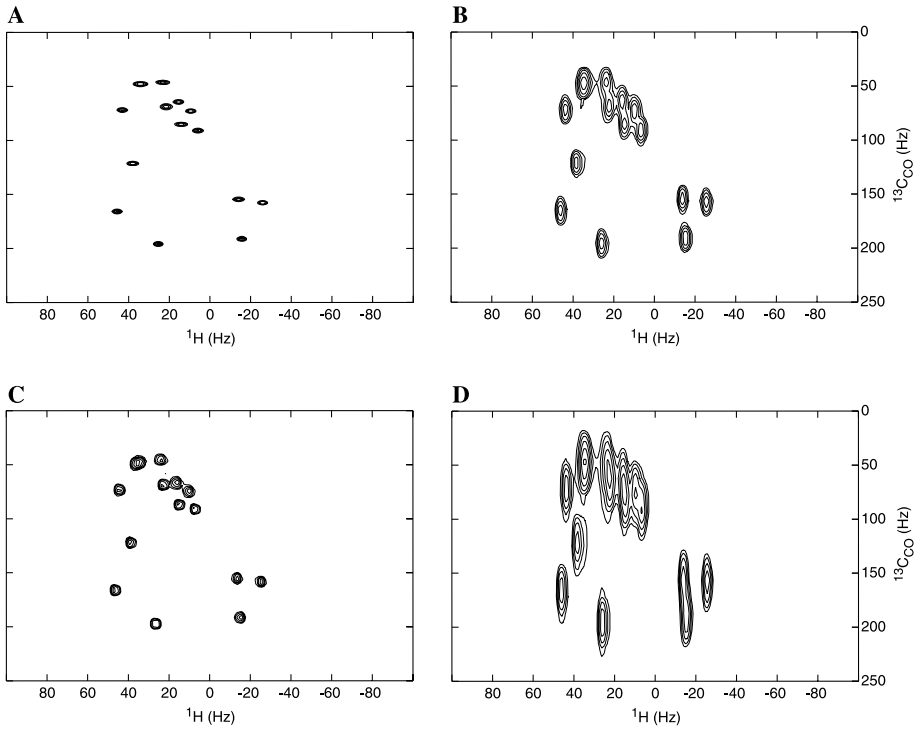

Fig. 3. Spectra that compare different processing methods for obtaining HMe, CMe, and CO correlations among nuclei of acetyl groups of the peracetylated nigeran oligosaccharide-alditol. Using the identical 3D data set ($512 \times 4 \times 16$ points), panels A, B, and D compare the resolution obtained after processing with the FDM (A), MI-LP (B), and FT (D), showing the HMe–CO projection. For comparison, a higher resolution FT spectrum from a longer (512×58 points, $\Delta_3 = 58.3$ ms) 2D experiment is shown in panel C. The FDM calculation was performed in a single window using $400 \times 4 \times 16$ points in the proton, methyl, and carbonyl dimensions, respectively. A regularization of $q = 0.005$ was applied with a smoothing of $\Gamma = 1$ Hz in each dimension. The results from processing the 3D experiment using the FDM are clearly in agreement with the longer 2D experiment processed with FT (C). However, a significant improvement in resolution was afforded in the indirect dimension of the 3D experiment by processing with the FDM (A) as compared to either the MI-LP (B) or FT (D).

numerous acetyl peaks. With this specific oligosaccharide, the presence of only 15 acetyl groups reduces the overlap and limits the signal required by the FDM. Therefore, while it may not be possible to treat every case with a 28 min 3D experiment, times in the order of 3–4 h are not unreasonable to obtain well-resolved spectra. Of course an experiment like this, coupled with FDM processing, opens the door to higher dimensional, high-resolution spectra. Experiments in four and possibly five dimensions may be developed in conjunction with FDM to yield a much larger amount of information from small data sets. While the 3D experiment presented here only demonstrates the transfer of magnetization through nuclei of the acetyl groups, this is the first building block of 4D experiments that can provide multi-dimensional correlations to the sugar ring proton resonances.

The results herein demonstrate the application of the 3D FDM to a group of molecules where its benefits are sorely needed. The extreme degree of spectral overlap in complex carbohydrates warrants the use of the highest number of dimensions and field strength humanly possible, perhaps more than any other macromolecules. Coupled with the use of acetyl and potentially other "isotags," more spin systems should be discernible and the determination of structures amid mixtures of oligosaccharides may be possible, reducing the need for tedious purification steps in the analysis of biological samples that often contain isomeric components. High-dimensional applications will also be relevant for NMR studies of other biological macromolecules, where the overlap of cross peaks remains a principal NMR restriction in extension to larger molecular sizes. At some point it will simply be necessary to employ additional dimensions to reduce overlap. One-dimensional methods such as FT or LP that must be applied sequentially to each dimension will reach ultimate limitations in these applications, as the experimental time required to acquire a reasonable number of increments in each added dimension will be prohibitive. Development of true multi-dimensional methods such as the FDM is essential if the goal of high-dimensional experiments is to be realized. While only the first step in this endeavor, we have now demonstrated a significant improvement over current standard 3D experiments using the FDM.

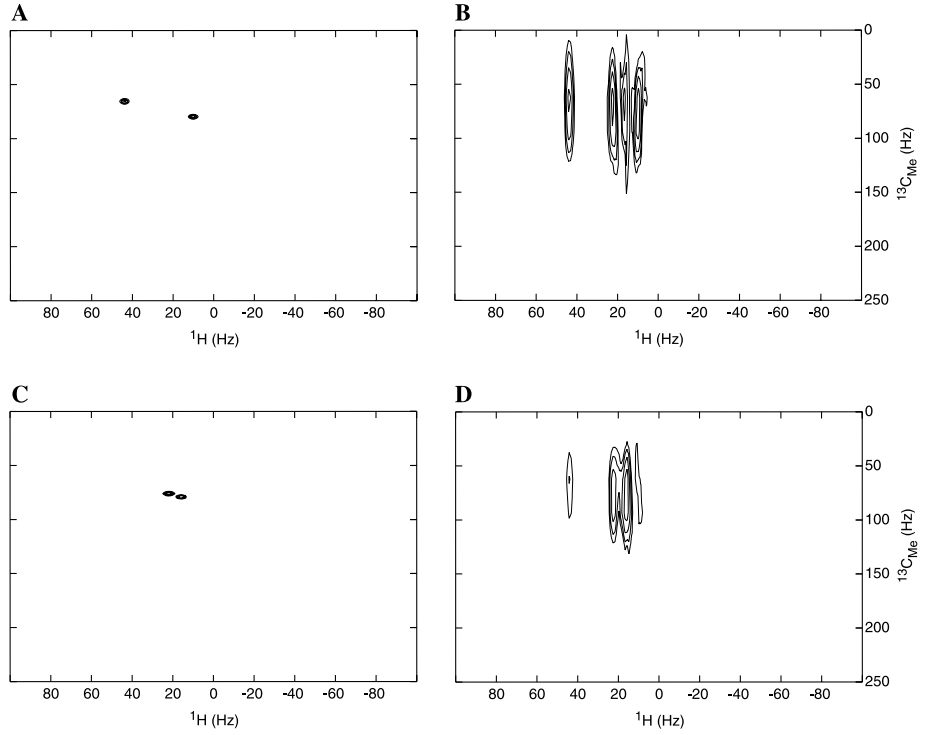

Fig. 4. Planes from a 3D spectrum correlating the HMe, CMe, and CO frequencies of acetyl groups of the peracetylated nigeran oligosaccharide-alditol. The identical data set was used as was shown in Figs. 2 and 3 ($512 \times 4 \times 16$ points), processed either with the FDM (panels A and C) or MI-LP (panels B and D). Planes A and B span the range from 63 to 70 Hz in the CO dimension, and planes C and D span the range 70–78 Hz. The FDM (panels A and C) resolves four independent signals into two sets, with two frequencies found on plane A and two on plane C. Estimates of the four frequencies by MI-LP processing, however (panels B and D), show much broader peaks, both in the CMe dimension and extending through both planes in the CO dimension. These frequency estimates were even broader using FT processing (not shown).

Acknowledgments

The authors thank Dr. Jianhan Chen, for both helpful discussions and the development of the 3D FDM. B.B. acknowledges NSF Grant MCB-0236103 and the Mizutani Foundation for Glycoscience. A.J.S. acknowledges the NSF for its support.

References

[1] K. Pervushin, R. Riek, G. Wider, K. Wuthrich, Transverse relaxation-optimized spectroscopy (TROSY) for NMR studies of aromatic spin systems in C-13-labeled proteins, J. Am. Chem. Soc. 120 (25) (1998) 6394–6400.

[2] G.S. Armstrong, J. Chen, K.E. Cano, A.J. Shaka, V.A. Mandelshtam, Regularized resolvent transform for direct calculation of 45 degree projections of 2D J spectra, J. Magn. Reson. 164 (2003) 136–144.

[3] V.A. Mandelshtam, H.S. Taylor, A.J. Shaka, Application of the filter diagonalization method to one- and two-dimensional NMR spectra, J. Magn. Reson. 133 (1998) 304–312.

[4] V.A. Mandelshtam, The multidimensional filter diagonalization method. I. theory and numerical implementation, J. Magn. Reson. 144 (2000) 343–356.

[5] J. Chen, V.A. Mandelshtam, A. Shaka, Regularization of the filter diagonalization method: FDM2K, J. Magn. Reson. 146 (2000) 363–368.

[6] J. Chen, A.A.D. Angelis, V.A. Mandelshtam, A.J. Shaka, Progress on two-dimensional filter diagonalization method an efficient doubling scheme for two-dimensional constant-time NMR, J. Magn. Reson. 162 (1) (2003) 74–89.

[7] V.A. Mandelshtam, FDM: the filter diagonalization method for data processing in NMR experiments, Prog. NMR Spectrosc. 38 (2001) 159–196.

[8] J. Chen, D. Nietlispach, V.A. Mandelshtam, A.J. Shaka, Ultra-high quality HNCO spectra with very short constant times, J. Magn. Reson. 169 (2004) 215–224.

[9] J.W. Homeister, M. Zhang, P.S. Frenette, R.O. Hynes, D.D. Wagner, J.B. Lowe, R.M. Marks, Overlapping functions of E- and P-selectin in neutrophil recruitment during acute inflammation, Blood 92 (1998) 2345–2352.

[10] E.F. Hounsell, M. Young, J. Davies, Glycoprotein changes in tumours: a renaissance in clinical applications, Clin. Sci. 93 (1997) 287–293.

[11] D.N.M. Jones, B. Bendiak, Novel multi-dimensional heteronuclear NMR techniques for the study of ^{13}C-O-acetylated oligosaccharides: expanding the dimensions for carbohydrate structures, J. Biomol. NMR 15 (1999) 157–168.

[12] B. Bendiak, T.T. Fang, D.N.M. Jones, An effective strategy for structural elucidation of oligosaccharides through NMR spectroscopy combined with peracetylation using doubly ^{13}C-labeled acetyl groups, Can. J. Chem. 80 (2002) 1032–1050.

[13] A. Bax, A.F. Mehlkopf, J. Smidt, Homonuclear broadband-decoupled absorption-spectra, with linewidths which are independent of the transverse relaxation time, J. Magn. Reson. 35 (1979) 373–377.

[14] K.E. Cano, M. Smith, A.J. Shaka, Adjustable, broadband, selective excitation with uniform phase, J. Magn. Reson. 155 (2002) 131–139.
[15] M.A. Smith, H. Hu, A.J. Shaka, Improved broadband inversion performance for NMR in liquids, J. Magn. Reson. 151 (2001) 269–283.
[16] D. Marion, M. Ikura, R. Tschudin, A. Bax, Rapid recording of 2D NMR-spectra without phase cycling—application to the study of hydrogen-exchange in proteins, J. Magn. Reson. 85 (1989) 393–399.
[17] A. Hammarström, G. Otting, Axial-peak artifacts in multipulse NMR experiments, J. Magn. Reson. Ser. A 109 (1994) 246–249.
[18] B. Bendiak, Nuclear magnetic resonance spectroscopy of peracetylated oligsaccharides having ^{13}C-labeled carbonyl groups in lieu of permethylation analysis for establishing linkage substitutions of sugars, Carbohydr. Res. 315 (1999) 206–221.
[19] F. Delaglio, S. Grzesiek, G.W. Vuister, G. Zhu, J. Pfeifer, A. Bax, NMRPipe: a multidimensional spectral processing system based on UNIX pipes, J. Biomol. NMR 6 (1995) 277–293.