Optimized Excitation and Automation for High-Resolution NMR Using B_1-Insensitive Rotation Pulses

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High-resolution NMR is a complex multidimensional technique in which the success of the experiment depends strongly on the careful optimization of many parameters. It would therefore be important for many application chemists and biologists if automated NMR were possible in which sensitivity and artifact reduction could be preoptimized. Recent advances using gradient technology have shown that spectral artifact reduction,[1] excellent water suppression,[1] and automated optimization (shimming) of the B_0-field homogeneity[2] are possible. However, despite the excellent quality of high-resolution NMR coils, sensitivity losses still occur due to inhomogeneities in the rf fields used for multinuclear excitation. Small losses for each individual rf pulse, especially when applying refocusing or inversion, may add up to large total losses for complex sequences. Adiabatic rf pulses[3] have the property of B_1-insensitivity, but until recently only adiabatic inversion and 90° excitation pulses were available. While important for high-quality decoupling,[4] adiabatic inversion pulses[3a,b] are not suitable to achieve refocusing in phase-sensitive high-resolution NMR because their single application induces a resonance-offset-dependent phase roll over the spectrum.[5] Although this phase roll is removed when two adiabatic full passage pulses are applied consecutively,[6] this approach lengthens the pulse sequence for each refocusing element and is disadvantageous for studying large macromolecules with short T_2-values. Recently, Garwood et al.[7] developed adiabatic rf pulses that can attain offset-independent wide band refocusing and excitation for arbitrary flip angles. In this paper we show that these B_1-insensitive rotation (BIR) pulses can be used as a substitution for most elements of multidimensional high-resolution NMR pulse sequences and result in optimum excitation by utilizing the complete coil volume and by reducing accumulated signal losses due to B_1-inhomogeneity over this volume. As a first example, we report a fully adiabatic fast heteronuclear-single-quantum-coherence sequence (BIR-FHSQC) and compare its performance to the hard-pulse FHSQC sequence.[8]

Figure 1 shows the general shape for a BIR pulse, and its principle mode of action is explained in the legend. Parts A and B of Figure 2 show the FHSQC and BIR-FHSQC pulse sequences, respectively. In general the hard pulses were directly substituted by BIR pulses[7a] of corresponding flip angle, but in some cases slight adjustments were made to retain the possibility of performing phase-sensitive 2D NMR while using the longer adiabatic pulses. For instance, two 180° ^{15}N refocusing pulses were used in t_1 to refocus ^{15}N chemical shift evolution during the finite proton 180° BIR pulse (504 μs). Since BIR pulses are self-refocusing for chemical shift evolution,[7a] time delays τ are of the same magnitude in the hard-pulse WATERGATE 3-9-19-19-9-3 sequence[1d] and the adiabatic WATERGATE section, without need to compensate for the finite length (504 μs) of the adiabatic pulses. We only found it necessary to adjust τ from 300 to 304 μs in the BIR sequence to achieve the best water suppression. We attribute this to possible small delays when reading out the complex pulses from the wave form board. While performing our experiments, we noticed that the BIR pulses could be implemented directly in the scalar coupling evolution delays without need to compensate for the finite length (504−512 μs) of the adiabatic pulses. This effect, which we intend to investigate in more detail, suggests that the particular BIR pulses used in this paper are self-compensating for scalar coupling evolution. Thus, two delays $\Delta = 1/(4J)$ were used during preparation, and exact refocusing was achieved by assuring that $2\delta + 5\tau = 1/(2J)$. Finally, the nitrogen pulses during preparation and refocusing are only necessary to invert the longitudinal spin component, and we therefore employed adiabatic full-passage pulses at these points.

All experiments were performed on a Bruker DMX 500 MHz instrument, equipped with a triple-frequency triple-gradient probe. The proton and nitrogen hard 90° pulse widths were 9.5 and 33 μs, respectively. To ascertain adiabaticity, all pulses in the BIR-FHSQC were 504 (^{1}H)−512 (^{15}N) μs, at the same power level as the hard pulses. The proton pulses were slightly shorter to compensate for a software delay when simultaneously playing out two amplitude- and phase-modulated rf pulses. Decoupling in the hard FHSQC sequence was performed using GARP. Adiabatic decoupling was achieved by using 2 ms adiabatic full-passage pulses modulated according to the MLEV-16 scheme.[4] The power level was 2.9 kHz for both cases, and decoupling was applied synchronously. After automated optimization of the B_0-field using adiabatic 1D field mapping,[2] spectra were obtained from a solution of 1.5 mM of doubly-labeled (^{15}N, ^{13}C) ribonuclease T1. Figure 3 compares 2D spectra and two 1D projections for the carefully optimized hard FHSQC (Figure 3A,C,E) and the automated BIR-FHSQC (Figure 3B,D,F) sequences. A signal gain of about 20% was achieved using the adiabatic sequence. This is the minimum gain, because the BIR-FHSQC sequence is insensitive to losses due to inaccurate flip-angle determinations and has to be optimized only once for each type of probe. We evaluated the origin of the signal increase by comparing the spatial excitation profiles for adiabatic and hard pulses. The results showed that

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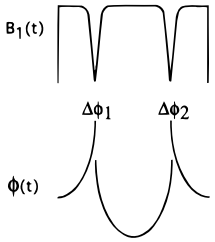

Figure 1. Amplitude-modulation and phase-modulation patterns for a B_1-insensitive rotation pulse with four 90° adiabatic segments (BIR-4).[7a] Since all segments are adiabatic, B_1-insensitive excitation is achieved. The adiabatic frequency sweep is achieved by applying a phase modulation corresponding to the time integral of the frequency sweep. If an rf pulse requires a certain phase offset, e.g. for phase cycling, this offset is added to the basic phase modulation. Arbitrary flip angles are achieved by applying intersegmental phase shifts $\Delta\phi_1 = -\Delta\phi_2 = 180° + \theta/2$, in which θ is the required flip angle.

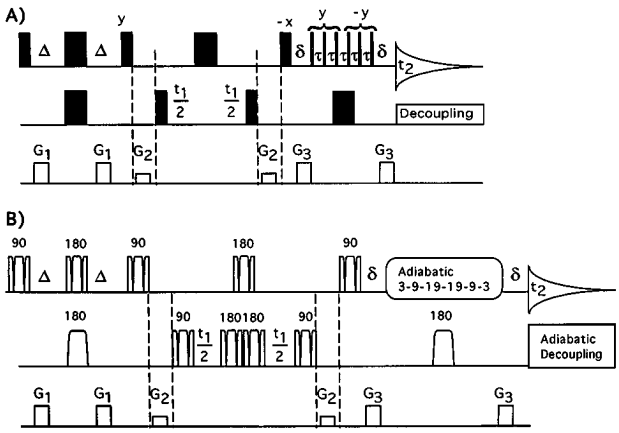

Figure 2. (A) Hard FHSQC and (B) BIR-FHSQC sequences. Wide and narrow rf pulses in A indicate hard 90° and 180° pulses, respectively. The WATERGATE 3-9-19-19-9-3 pulses are narrower than in reality, where their length is proportional to their index number. The pulse phases are *x*, unless indicated, and are equal in A and B. Hard and adiabatic decoupling used GARP and MLEV-16 modulation, respectively. (see text). Time delays for scalar coupling evolution are related by $\Delta = \delta + 2.5\tau = 2.8$ ms; τ was 304 and 300 μs in the BIR-FHSQC and FHSQC sequences, respectively. Gradient strengths (*x*; *y*; *z*) were $G_1 = G_3 = 110$; 220; 150 mT/m and $G_2 = 66$; 66; 90 mT/m. In B, the ^{15}N 180° pulses during preparation and refocusing are adiabatic full-passage pulses. All others are BIR-4 pulses.

the adiabatic pulses excite a slightly larger volume (explaining about 10% of the signal increase) and, contrary to the hard pulses, essentially do not accumulate losses due to B_1-homogeneity when applying a series of rf pulses (explaining the residual signal increase). Finally, upon switching protein samples, we were able to directly run the adiabatic sequence with maximum sensitivity without adjusting for small changes in tuning and matching.

In summary, we have shown that the use of special B_1-insensitive rotation (BIR) rf pulses can achieve optimum

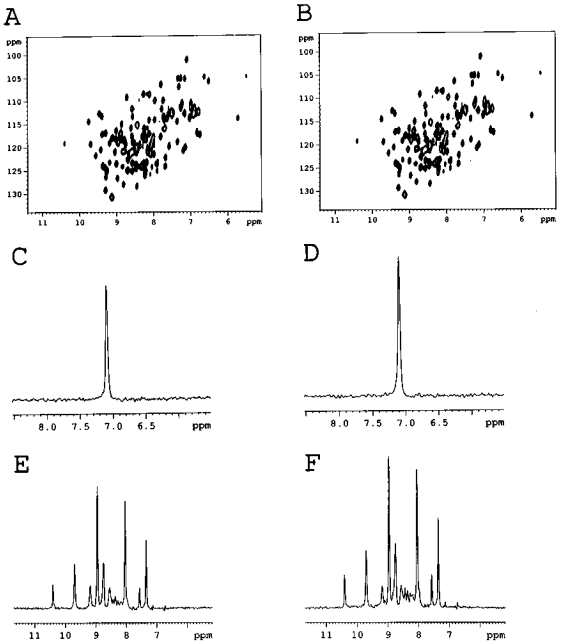

Figure 3. 2D spectra and individual slices for the ^{15}N FHSQC (A, C, E) and BIR-FHSQC (B, D, F) sequences applied to 1.5 mM doubly-labeled ribonuclease T1 in 90%/10% H_2O/D_2O. Spectral acquisition took 10.5 min. for 128 increments (four scans) with TPPI and two-step phase-cycling (first 90° ^{15}N pulse: *x*, $-x$). The acquisition time was 128 ms, the interscan delay was 1 s; 1K complex points were acquired in t_2; spectral widths were 50 and 16 ppm in t_2 and t_1, respectively. The final data were zero filled to 1K × 1K. Spectra were base line corrected to reduce the small residual water signal. Sine-bell square apodization was used in t_2 and t_1, respectively. All spectra were processed equally, and equal receiver gains were used. The slice positions correspond to the maximum peak intensity of the leftmost peak at 101 (C, D) and 119 ppm (E, F).

excitation in multidimensional NMR. Since the accuracy of the flip angles depends only on the fulfillment of the adiabatic condition (minimum power level), the experiments can be automated and a single pulse length per rf channel can be used for all different flip angles. Adiabatic pulses are often affiliated with power deposition problems. However, almost all power deposition for the experiment comes from decoupling (128 ms), for which the average adiabatic power is actually lower than the average hard power, and we have experienced no power deposition problems. We foresee that, in addition to complete automation, even higher signal gains can be achieved in the complex multipulse multidimensional heteronuclear experiments presently used in high-resolution NMR.

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