Communications to the Editor

Exchangeable Proton NMR without Base-Line Distortion, Using New Strong-Pulse Sequences

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Pulsed nuclear magnetic resonance of exchangeable protons in H_2O solvent is most often accomplished by using Redfield's "2-1-4" weak-pulse sequence, which leaves the water proton magnetization unperturbed and provides second-order cancellation: the amplitude of a peak is a quadratic function of its distance to the frequency of cancellation.[1,2] Hence, solvent suppression is insensitive to slight errors and drifts in frequency or field-gradient correction. The same features can be achieved by a sequence[3] of strong, nonselective pulses (15°, τ, 15°, 15°, τ, 15°). The advantages of strong-pulse irradiation lie in the easier designing of sequences, in their insensitivity to long-term drift in pulse amplitude, and in the simpler generation of such pulses in an FT spectrometer.

A defect of the sequences mentioned above is the generation of phase shifts, which combined with the existence of a residual but still intense solvent peak, lead to base-line undulation.[3] We have searched for strong-pulse solvent-suppressing sequences that would produce no phase shift and hence no base-line undulation. Two sequences will be presented here. They share the following properties: (a) only strong, nonselective pulses (10 W) are used; (b) the pulse is at the solvent frequency; (c) they use only two phases, 0° and 180°; (d) no linear phase correction is required. The first sequence ("jump and return") provides first-order cancellation of the solvent peak. The phase is strictly constant across the spectrum, except for a step change of 180° at the solvent frequency. The second sequence ("set, jump, and return") gives second-order cancellation and only small phase shifts.

The JR ("Jump and Return") Sequence (90°$_y$, τ, 90°$_{-y}$). The first pulse brings all spins along $0x$ in the rotating frame. During the waiting time they fan out in the xy plane; the solvent spins are at resonance and remain along $0x$. The waiting time determines the variation of amplitude with frequency and the final longitudinal magnetization. The second pulse brings all spins back from the $x0y$ plane to positions in the $z0y$ plane, the solvent being returned to $0z$. The free precession is then recorded. Transverse magnetizations start along $0y$ on one side of the solvent peak, along $-0y$ on the other.

Tuning is done while observing the free precession. It consists in (a) adjusting the pulse length to 90° (an error will only give small phase shifts, it will not affect solvent cancellation), (b) correcting for pulse-phase errors by a slight change in radiofrequency (rf) or by an adjustable phase differential, (c) correcting for pulse inequality (and/or radiation damping) by an adjustable pulse length differential.

Figure 1 shows a spectrum of yeast tRNA[Phe] obtained with the JR sequence. It exhibits the characteristic 180° phase difference between the two sides of the solvent peak. Good phase control and shaping of the rf pulses are involved in these experiments. In our spectrometer, all rf manipulations are carried out at the
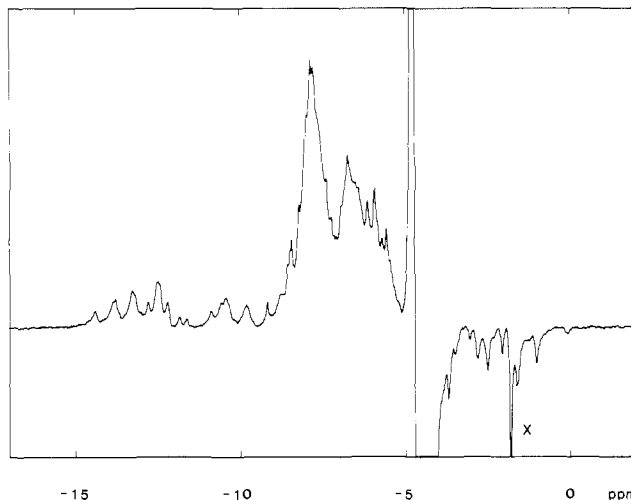

Figure 1. 276-MHz JR spectrum of yeast tRNA[Phe]. Conditions: 0.9 mM tRNA; 35 °C; 150 FIDs; waiting time τ between the two pulses of the JR sequence, 125 μs; repetition time, 0.2 s; broadening, 5 Hz. No phase correction has been applied except for audio-filter response. The water peak (at −4.65 ppm) is reduced 500-fold. X is the cacodylate buffer. Note the good signal-to-noise ratio.

intermediate frequency of 10 MHz.[4]

Water is reduced by a factor of 300 on each free precession, similar to what we obtain with 2-1-4 type sequences. It is apparently limited by phase fluctuations of the rf pulses. Phase hum or fluctuations of the generator show up clearly in the free precession of a strong signal, at resonance, in the dispersion mode. They give deviations from zero. Hum in the field shimming coil shows up similarly.

The SJR ("Set, Jump, and Return") Sequence ($\theta°_{-y}$, 10τ, $\theta°_y$, JR). The JR sequence provides first-order cancellation only. Sequences providing second-order cancellation without phase-shift are unknown to us; they may be restricted by mathematical considerations. The SJR sequence gives second-order cancellation at the cost of modest phase shifts.

We start with a pulse of tilt-angle $\theta = 5.7°$ (i.e., $\sin \theta = 0.1$) along $-0y$ and wait for 10 times longer than the JR waiting time. During this time, all spins precess on a small circle of axis $0z$. Spins that are at resonance do not move. Those that are near enough for a linear approximation to be valid spread out along the tangent (parallel to the $0y$ axis) by the same amount as they will during the waiting time of the upcoming JR sequence, *but in the opposite sense*. Hence they will be refocused by JR.

The range of second-order cancellation is changed by varying the "set" angle θ, the first waiting period being set to $(1/\sin \theta)$ times the JR one. Spins further away in frequency precess all around the small circle. This will modify their position at the end of the sequence, inducing phase shifts. These are large close to the solvent but decrease and oscillate as one goes away from the solvent frequency. Figure 2 compares the JR and SJR spectra.

SJR has been used for various NOE experiments in which selective line inversion is also obtained by the use of strong pulses (in preparation). Pulse sequences devoid of phase-shifts could be particularly useful in 2-D NMR of exchangeable protons. Respect of spectral amplitudes is also important in 2-D NMR. The sine amplitude response of the JR sequence (and SJR as well,

(1) Redfield, A. G.; Kunz, S. D.; Ralph, E. K. *J. Magn. Reson.* **1975**, *19*, 114–117.

(2) Redfield, A. G.; Kunz, S. D. "NMR in Biochemistry"; Opella, S. J., Lu, P., Eds.; Marcel Dekker: New York, 1979; pp 225–239.

(3) Plateau, P.; Dumas, C.; Guéron, M., manuscript in preparation.

(4) Caron, F.; Guéron, M.; Nguyen Ngoc Quoc Thuy; Herzog, R. F.; *Rev. Phys. Appl.* **1980**, *15*, 1267–1274.

Figure 2. Demonstration of the spectral response in the JR and SJR sequences. The NMR lines from an H_2O sample are obtained for different offsets (indicated in Hz) between the proton Larmor frequency and the excitation frequency. (A) Coarse representation of the response to an SJR sequence ($\theta = 5.7°$, $\tau = 125$ μs). The small phase shifts described in the text are observed. Apart from these, a JR sequence would give the same results on this scale. The gain has been reduced by a factor 10 as compared to B and C. (B) The response to the same SJR sequence, in the range of small offsets. Notice the extensive cancellation, up to ± 20 Hz; a dispersion component emerges first as offset is increased. (C) The small-offset response to a JR sequence ($\tau = 125$ μs). The amplitude is a linear function of offset and the mode is pure absorption for all offset values.

except quite close to the solvent frequency (Figure 2A)) may then be valuable.

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Fast Hydrolysis of Alkyl Radicals with Leaving Groups in the β Position

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Upon reaction of radiolytically generated[1] OH radicals with alkyl bromides, alkyl chlorides, trialkyl phosphates, alkyl methanesulfonates, or dialkyl sulfates in aqueous solution, all possible H-abstraction radicals are produced (see, e.g., eq 1).

Some of these radicals give rise to rapid formation of acid (see Table I), as was found by time-resolved conductivity measurements upon pulse radiolysis.[2,3] The acid-forming radicals were identified in some instances as the β-substituted ones by specifically producing them, e.g., by reacting radiolytically generated[1] hydrated electrons, e_{aq}^-, with vicinal dihaloalkanes[4,5] (see e.g., eq 2).

(1) Upon radiolysis of water, OH radicals and hydrated electrons, e_{aq}^-, are the main radical species formed. In the presence of N_2O the latter are transformed into OH radicals: $N_2O + e_{aq}^- \rightarrow OH\cdot + OH^- + N_2$. *tert*-Butyl alcohol was added instead of N_2O in order to observe the reaction of e_{aq}^- with substrate. See: Henglein, A., Schnabel, W., Wendenburg, J., Eds. "Einführung in die Strahlenchemie"; Verlag Chemie: Weinheim, 1969.

(2) For the technique and basic chemical considerations see: Klever, H. Ph.D. Thesis, Ruhr-Universität, Bochum, 1974. Applictions may be found in ref 9 and 14.

Table I. First-Order Rate Constants of Acid Formation Observed by Time-Resolved[a] Conductivity Measurements upon Pulse Radiolysis[1,2] of N_2O-Saturated Aqueous Solutions of the Substrates Listed Below (10^{-3}–10^{-2} M) at pH 4.5–5 and 292 ± 3 K

substrate	radical undergoing hydrolysis[b]	rate constant of acid formation, s^{-1}
1-chloropropane		*c*
1- or 2-bromopropane[d]		7×10^3
1-propylmethanesulfonate		2×10^5
di-1-propyl sulfate		$\geq 10^6$
1-chlorobutane		*c*
1-bromobutane		
1-butylmethanesulfonate		$\geq 10^6$
2-chlorobutane		3.5×10^5
2-bromobutane		$\geq 10^6$; 10^4
1-chloro-2-methylpropane		3.5×10^4[f]
methyl bis(2-methyl-1-propyl) phosphate		3×10^4
tris(2-methyl-1-propyl) phosphate		1.4×10^4
2-methyl-1-propyl-methanesulfonate		$\geq 10^6$
chlorocyclohexane		$\geq 10^6$

[a] The H-abstraction radicals are produced within the pulse duration of 1 μs. The rate constants were independent of dose rate (accuracy $\pm 25\%$). Time resolution 10^{-6} s. [b] The radicals of this column are the most abundant ones among the β-substituted alkyl radicals; whether these radicals are in equilibrium with the isomeric radicals resulting from reverse 1,2-leaving-group shifts and the latter contribute to the hydrolysis remains an open question. [c] No acid formation; the first half-life of the second-order termination of radicals was mainly in the range 10^{-3}–10^{-2} s after the pulse, and the first-order decay constant of the radical is therefore $<10^2$ s^{-1}. [d] The H-abstraction radical XII (Chart I) undergoes a 1,2-Br shift to yield XIII prior to hydrolysis in a very fast reaction.[12] [e] Two processes were observed, one fast and unresolved, assigned from analogies to the hydrolysis of the first radical; the second radical is expected from the 1,2-Br shift[13] XIV $\rightarrow$ XV, and therefore its rate of hydrolysis is identical with the one observed after H abstraction from 1-bromobutane. [f] Accuracy $\pm 5\%$.

The reaction of 2,3-dichlorobutane with e_{aq}^- leads to a fast ($k \geq 10^6$ s^{-1}) and a slower ($k = 3.5 \times 10^5$ s^{-1}) increase of conductivity. The fast one is assigned to reaction 2 and the slow one to the decay of the radical formed in that reaction. The slower kinetics was

(3) We have also determined the acid formed upon ^{60}Co γ irradiations at low conversion of substrate by titration of H^+, Cl^-, or Br^- with use of ion-selective electrodes; the yields of acid/OH· were the same as derived from the size of the conductivity changes.

(4) The reaction VIII (Chart I) + $e^- \rightarrow Cl_2^- \cdot$ + butenes, as observed in frozen methanol by Mishra and Symons (Mishra, S. P.; Symons, M. R. C. *Tetrahedron Lett.* **1975**, *30*, 2597) was not observed in the aqueous phase.

(5) γ-Bromo- or γ-chloroalkyl radicals produced analogously did not lead to acid formation; α-bromo- or α-chloroalkyl radicals produced in this fashion did not lead to acid formation in most cases, although some of them gave rise to rather little formation of acid in the millisecond time range.