JOURNAL OF MAGNETIC RESONANCE 76, 174–177 (1988)

Resolution-Enhanced Correlation of ^1H and ^{31}P Chemical Shifts

DANIEL WILLIAMSON AND AD BAX

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

Received July 29, 1987

A variety of methods for correlating ^1H and ^{31}P chemical shifts have been proposed in recent years (*1–6*). In our experience, best results (considering both resolution and sensitivity) are obtained with the relatively simple scheme of Fig. 1a (*5*). This scheme is essentially the ^1H-detected version of the oldest and simplest heteronuclear correlation scheme (*7*), yielding pure absorption spectra. Each of the four components of the correlation multiplets in the 2D spectrum displays the ^1H–^1H multiplet structure in the F_2 dimension, and the ^1H–^{31}P multiplet structure (except for the active coupling) in the F_1 dimension. The four components are in antiphase in the regular manner, $\begin{smallmatrix}+ & -\\ - & +\end{smallmatrix}$, and the antiphase components are separated by the active J_{PH} coupling. In the F_2 dimension of the spectrum, the total multiplet width is equal to the sum of all the couplings experienced by the proton. Similarly, in the ^{31}P dimension, the multiplet width equals the sum of all couplings experienced by the ^{31}P nucleus. This communication describes a general way to decrease the total multiplet width in the F_1 dimension to the size of the J_{PH} coupling that is responsible for the cross peak, i.e., a method for suppressing all passive couplings in the F_1 dimension of the 2D spectrum.

The new pulse scheme is sketched in Fig. 1b. A 120° ^1H pulse is inserted in the center of the evolution period of the standard scheme (Fig. 1a). Essentially, this 120° pulse acts partially as a 180° pulse and flips the spin state of 75% of the protons. This means that for a ^{31}P nucleus that is coupled to only one single proton, 75% of the ^{31}P nuclei will not show any dephasing due to ^1H–^{31}P coupling at the end of the evolution period, whereas 25% (for which the ^1H has not been flipped) will experience the full ^1H–^{31}P dephasing during the evolution period. This latter component is the one that gives rise to the correlation multiplet in the 2D spectrum (at 25% of the regular intensity). If the ^{31}P is coupled to two protons, 75% of the magnetization that has dephased during the evolution period as the result of coupling to one of the protons will not show dephasing due to coupling to the second proton; i.e., the coupling to the second proton will not appear in 75% of the cross-peak contributions, resulting in a significant narrowing of the multiplet structure in the F_1 dimension of the 2D spectrum. A more exact analysis for the three-spin system, I_1I_2S, where I_1 and I_2 are protons and S is the ^{31}P nucleus, is presented below.

In the calculations below, the flip angle of the pulse at the center of the evolution period is set to α. To eliminate any heteronuclear multiple-quantum coherence created by this $\alpha(^1H)$ pulse, its phase is cycled along all four axes without changing the receiver

0022-2364/88 $3.00

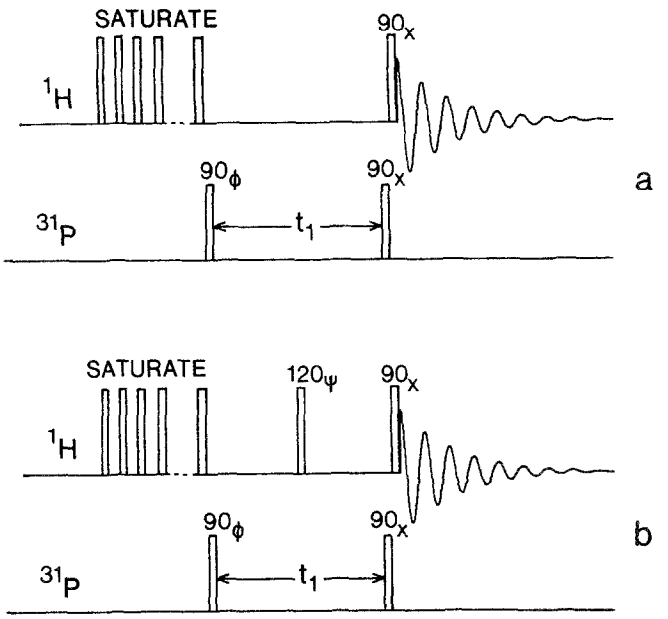

FIG. 1. Pulse scheme of (a) the regular ^{1}H-detected ^{31}P–^{1}H correlation scheme and (b) the new sequence that employs partial decoupling of passive spins in the F_1 dimension. The phase ϕ is cycled according to $\phi = x, y, -x, -y$, with the receiver +, +, −, −, and data for odd and even numbered scans are stored separately. To suppress heteronuclear multiple-quantum artifacts in scheme (b), the phase ψ is incremented by 90° after every four scans, without changing the receiver reference phase.

reference phase. Using operator formalism (*8*) and omitting the effect of chemical shifts, one finds for the ^{31}P magnetization at the end of the evolution period

$$S_x \xrightarrow{H_J t_1/2;\ \alpha(I);\ H_J t_1/2} a^2 S_x + ab(2S_y I_{1_z} s_1 + S_x c_1) + ab(2S_y I_{2_z} s_2 + S_x c_2) - b^2(4S_x I_{1_z} I_{2_z} s_1 s_2 - 2S_y I_{1_z} s_1 c_2 - 2S_y I_{2_z} c_1 s_2 - S_x c_1 c_2) \quad [1]$$

with $a = (1 - \cos\alpha)/2$; $b = (1 + \cos\alpha)/2$; $s_1 = \sin(\pi J_{I_1 S} t_1)$; $s_2 = \sin(\pi J_{I_2 S} t_1)$; $c_1 = \cos(\pi J_{I_1 S} t_1)$; $c_2 = \cos(\pi J_{I_2 S} t_1)$; and H_J denotes the scalar coupling part of the Hamiltonian.

The terms in expression [1] containing the coefficient b^2 are the terms that occur in the regular scheme of Fig. 1a, where $b = 1$. The terms containing coefficient ab are the terms corresponding to S spins that experience coupling to only one of the I spins during the evolution period. These are the desired terms giving rise to the narrow multiplet width in the F_1 dimension of the 2D spectrum. The larger the coefficient ab, the higher the sensitivity of the experiment. On the other hand, the terms containing b^2 must be kept small since these give rise to the broad F_1 multiplet structure. In practice, a flip angle α of 120° is a good compromise for ^{31}P nuclei coupled to 2–4 protons. Because $ab \ll 1$, one might expect a dramatic loss in sensitivity for this experiment compared to the regular scheme of Fig. 1a. However, for a ^{31}P nucleus coupled to N protons, the multiplet intensity in this experiment is spread in the F_1

dimension over only two components, instead of 2^N components, which largely negates the loss in signal-to-noise ratio.

The method is demonstrated for a sample of the oligonucleotide d(CGCGAATTCGCG)$_2$, p^{2}H 7.0, 100 mM NaCl, 400 OD_{260} units in 0.5 ml D_2O. Spectra were recorded at 38°C, on a modified NT-270 spectrometer equipped with a Cryomagnet Systems magnet and probe. Spectra obtained with the two schemes of Fig. 1 are shown in Fig. 2. Both spectra were recorded and processed with identical parameters. Each spectrum results from a 2 × 55 × 512 data matrix, with acquisition times of 330 and 132 ms in the t_1 and t_2 dimensions, respectively. Five hundred twelve scans were recorded per t_1 value and the measuring time was 12 h per spectrum. The lowest contour level in Fig. 2a is drawn at a level 40% lower than the lowest level in Fig. 2b, which indicates that the sensitivity in Fig. 2a is about 40% lower than that obtained with the original method. In fairness, it should be noted that the conditions under which the spectra were recorded were optimized for the highest F_1 resolution. If conditions were optimized for sensitivity, by choosing a shorter t_1 acquisition period and less severe resolution enhancement in the F_1 dimension, the difference in signal-to-noise ratio would be considerably larger than 40%. As can be seen from Fig. 2, the spectral resolution has improved dramatically by using the scheme of Fig. 1b and the

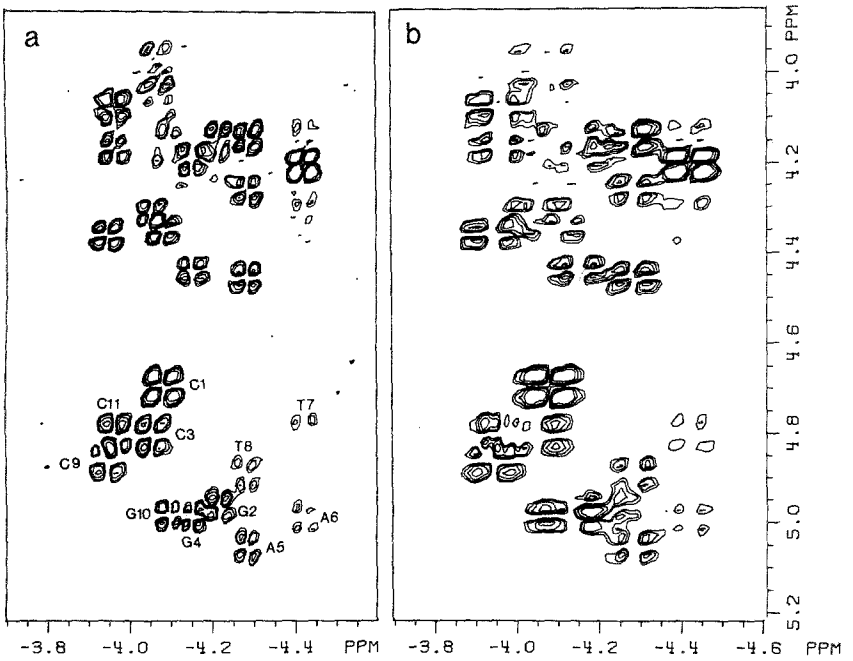

FIG. 2. Comparison of ^{1}H–^{31}P correlation spectra of d(CGCGAATTCGCG)$_2$ recorded with (a) the method of Fig. 1b and (b) the method of Fig. 1a. The spectra are absorptive in both dimensions and both negative and positive resonances are plotted. Each spectrum results from a 2 × 55 × 512 data matrix, with 512 transients per t_1 value; measuring time 12 h per spectrum. The spectra are recorded at 270 MHz ^{1}H frequency. The lowest contour level in (a) is drawn at an intensity level that is 40% lower compared to spectrum (b). Assignment of the C3′H resonances, based on 2D NOE spectra and on ^{31}P–C4′H correlation, is given in Fig. 2a.

extensive overlap present in the C3′H–^{31}P correlations in Fig. 2b is removed almost completely.

It is clear from the above that a substantial gain in F_1 resolution can be obtained by inserting the less than 180° pulse at the center of the evolution period. The gain in resolution is most pronounced at lower fields, where the F_1 multiplet width is dominated by the passive J_{PH} couplings. At higher fields, the chemical-shift anisotropy contribution to the ^{31}P T_2 relaxation becomes more significant and will eventually dominate the resolution in this dimension. For the dodecamer d(CGCGAATT-CGCG)$_2$, the ^{31}P T_2 value decreases from about 125 milliseconds in a 270 MHz spectrometer to about 70 milliseconds in a 500 MHz spectrometer. Nevertheless, even at 500 MHz ^{1}H frequency, a significant improvement in F_1 resolution over the original method was obtained with the scheme of Fig. 1b (data not shown). As shown elsewhere (*6*), spectra similar to Fig. 2a but with much higher sensitivity can be recorded for the C3′H spectral region by using a selective 180° ^{1}H pulse at the center of the evolution period. However, this method is not applicable to the typically very crowded region of the C4′ and C5′ protons.

ACKNOWLEDGMENT

We thank Rolf Tschudin for continuing technical support.

REFERENCES

1. P. H. BOLTON, *in* "Biomolecular Stereodynamics" (R. H. Sharma, Ed.), Vol. 2, p. 437, Adenine Press, New York, 1981.
2. M. H. FREY, W. LEUPIN, O. W. SØRENSEN, W. A. DENNY, R. R. ERNST, AND K. WÜTHRICH, *Biopolymers* **24,** 2371 (1985).
3. D. G. GORENSTEIN, D. LAI, AND D. O. SHAH, *J. Am. Chem. Soc.* **106,** 6717 (1984).
4. R. A. BYRD, M. F. SUMMERS, G. ZON, C. SPELLMEYER FOUTS, AND L. G. MARZILLI, *J. Am. Chem. Soc.* **108,** 504 (1986).
5. V. SKLENAR, H. MIYASHIRO, G. ZON, H. T. MILES, AND A. BAX, *FEBS Lett.* **208,** 94 (1986).
6. V. SKLENAR AND A. BAX, submitted for publication.
7. A. A. MAUDSLEY AND R. R. ERNST, *Chem. Phys. Lett.* **50,** 368 (1977).
8. R. R. ERNST, G. BODENHAUSEN, AND A. WOKAUN, "Principles of Nuclear Magnetic Resonance in One and Two Dimensions," p. 25, Clarendon Press, Oxford, 1987.