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Novel ^{1}H-^{31}P heteronuclear coherence transfer spectroscopy based on spin locking MLEV-17 and its application to signal assignment for a short DNA fragment

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We have applied the MLEV-17 proton spin locking pulse to ^{1}H-^{31}P 2D heteronuclear correlation spectroscopy. Using this technique, long-range correlations between a proton and phosphorus that is up to 5 bonds distant are observed. Thus, the 3′-, 4′- and 5′-proton resonances can be traced 'sequentially' along the chain on the 2D NMR chart. Application of this technique to the complete assignment of the sugar proton and phosphorus resonances for d(ApGpA) is reported. This technique can also be used for convenient assignment of the phosphorus resonances of oligonucleotides as correlations between the 1′-proton and its 3′-side phosphorus are observed.

NMR, 2D; NMR, ^{31}P-; Coherence transfer spectroscopy; Oligonucleotide; Resonance assignment

1. INTRODUCTION

The ^{31}P NMR study of nucleic acids can provide information on the local geometries of the sugar-phosphate backbone. Assignment of the resonances is carried out in ^{31}P-detected [1–4] or ^{1}H-detected [5] ^{1}H-^{31}P heteronuclear shift correlation experiments that are based on spin coupling between phosphorus and protons. However, all these methods usually give only one correlation per phosphorus with each sugar proton.

Here, we report a complete assignment of the phosphorus and proton resonances for d(ApGpA) by using ^{1}H-^{31}P heteronuclear coherence transfer spectroscopy with proton spin locking. In the 2D spectrum, the 3′-, 4′- and 5′-protons of the internal residue (-pGp-) gave two cross-peaks with both the 3′- and 5′-side phosphorus atoms. Therefore, we can trace the proton resonances of each sugar position 'sequentially' along the chain on the 2D NMR chart, and can assign all the sugar proton resonances without observation of NOEs. In addition, this pulse also provides cross-peaks between the 1′-proton and its 3′-side phosphorus, making assignment of the ^{31}P resonances very easy.

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Abbreviations: 2D, two-dimensional; NOE, nuclear Overhauser effect; A_{260}, absorbance at 260 nm; FID, free induction decay

2. MATERIALS AND METHODS

d(ApGpA) was synthesized by the phosphotriester method. The NMR sample was prepared by dissolving 75 A_{260} units (5.3 mM) of d(ApGpA) in D_2O (0.4 ml) containing 100 mM NaCl and 10 mM sodium phosphate buffer (pH 6.8).

NMR spectra were recorded on a Jeol GX-500 (500 MHz for ^{1}H, 202 MHz for ^{31}P) spectrometer with a 5 mm proton probe and an optional 5 mm phosphorus probe at 30°C. Proton chemical shifts are measured relative to internal *tert*-butanol (1.23 ppm). Phosphorus chemical shifts are relative to external trimethyl phosphate (10% in ethanol). The pulse sequences used are shown in (cf. fig.1.

A ^{1}H-^{1}H coherence transfer spectrum was recorded with MLEV-17 [6] for a mixing time of 70 ms. 16 FIDs of 1024 points were accumulated at 128 t_1 values (fig.2). For both dimensions, the observation range was 5000 Hz, and ^{1}H (90°) pulse width was 39.5 μs.

^{1}H-^{31}P coherence transfer spectra with MLEV-17 (fig.1D) were recorded for mixing times (τ_m) of 30 and 80 ms (fig.3A,B).

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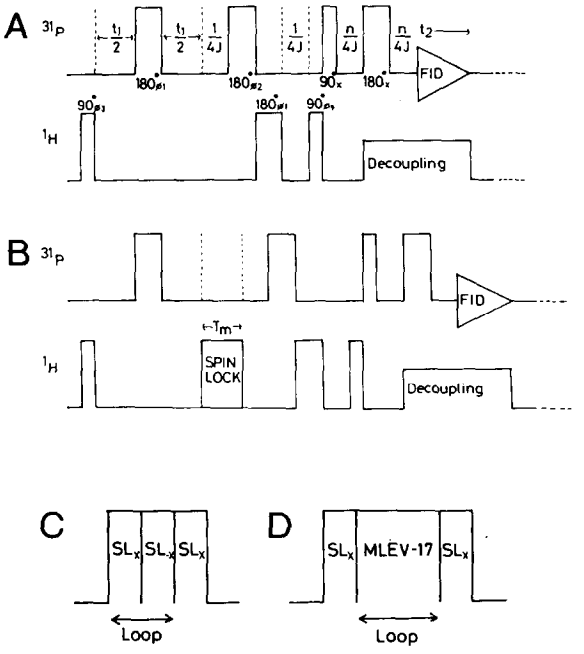

Fig.1. Pulse sequences for ^{1}H-^{31}P heteronuclear coherence transfer spectroscopy. (A) Pulse sequence for coherence transfer spectroscopy extended from INEPT. (B) Pulse sequence with proton spin locking. Spin locks using the phase-alternated pulse cycle (C) and using the MLEV-17 (D). A J value of 12.5 Hz and $n = 1$ were used. Combinations of the pulse phases, ϕ_1 and ϕ_2, were (x,x), $(-x,-x)$, $(x,-x)$ and $(-x,x)$. Each combination was repeated four times and cycled in this order. ϕ_3 was cycled $x,-x,x,-x$. ϕ_4 was cycled $x,x,-x,-x$.

^{31}P detection was performed on the carrier frequency at 202.35 Hz and a 2000 Hz range was observed. 16 FIDs of 1024 points were accumulated at 128 t_1 values. The observation range was 5000 Hz in t_1 for protons. Pulse widths were 40.0 μs for ^{1}H (90°) and 8.5 μs for ^{31}P (90°). The spin lock (SLx) time was 5 ms in both MLEV-17 and the phase-alternated spin lock [7] (cf. fig.1).

Before Fourier transformation, a slightly shifted gaussian function window for t_2 and an exponential window for t_1 were applied, and the t_1 dimension was zero-filled four times (to 512 points). All 2D data were collected and processed in the 'phase-sensitive' mode.

3. RESULTS AND DISCUSSION

2D homonuclear coherence transfer spectroscopy with MLEV pulse cycles [6,8] is effective for the investigation of macromolecules such as proteins [6,9] and nucleic acids [10]. In the case of oligonucleotides, it is useful for determination of the intra-residual spin connectivities of deoxyribose protons. Three sets of protons which belong to the same sugar residue of d(AGA) can be defined by this method [6] (fig.2), but the positions of the residues in the trimer are not obvious.

Applying ^{1}H-^{31}P heteronuclear coherence transfer spectroscopy with MLEV-17, we can trace the cross-peaks for a set of protons (3′, 4′ or 5′) along the chain on the 2D NMR chart (fig.3B), since the signals for the protons of two adjacent residues can be connected through the phosphorus between them.

When a mixing time of 80 ms is used, the 3′, 4′ and 5′-protons of the central residue, 2G, give two cross-peaks with both the 5′- and 3′-side phosphate (fig.3B, see also fig.4). The 3′, 4′ and 5′-protons of the terminal residues, 1A and 3A, give a cross-peak with either the 3′- or 5′-side phosphate. The cross-peak connectivity can be traced sequentially along the chain in each set of 3′, 4′ and 5′-protons. It is particularly clear in the case of the 3′-proton (H3′). The H3′ of 2G gives a strong correlation with the proximal phosphate (P_{II}) and a weak correlation with the distant phosphate (P_I). Thus, the cross-peaks can be traced by the following motif 'strong (1AH3′-P_I) – weak (P_I-2GH3′) – strong (2GH3′-P_{II}) – weak (P_{II}-3AH3′)' from the 5′-terminus (fig.3B, see

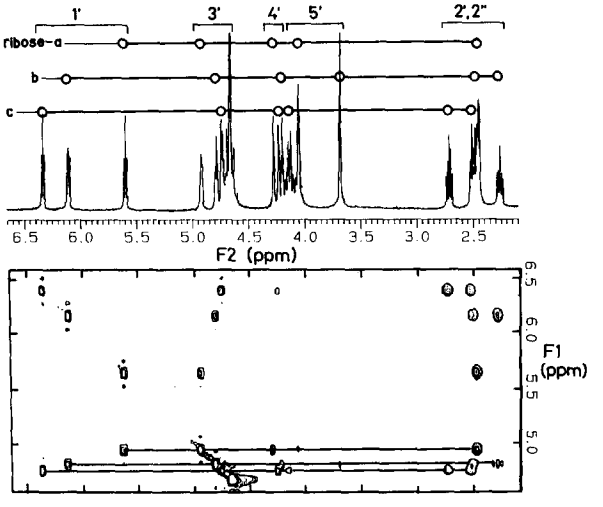

Fig.2. Expanded region of the ^{1}H 2D coherence transfer spectrum of d(AGA) with MLEV-17 and a mixing time of 70 ms. Intra-residual connectivities are indicated above the normal spectrum.

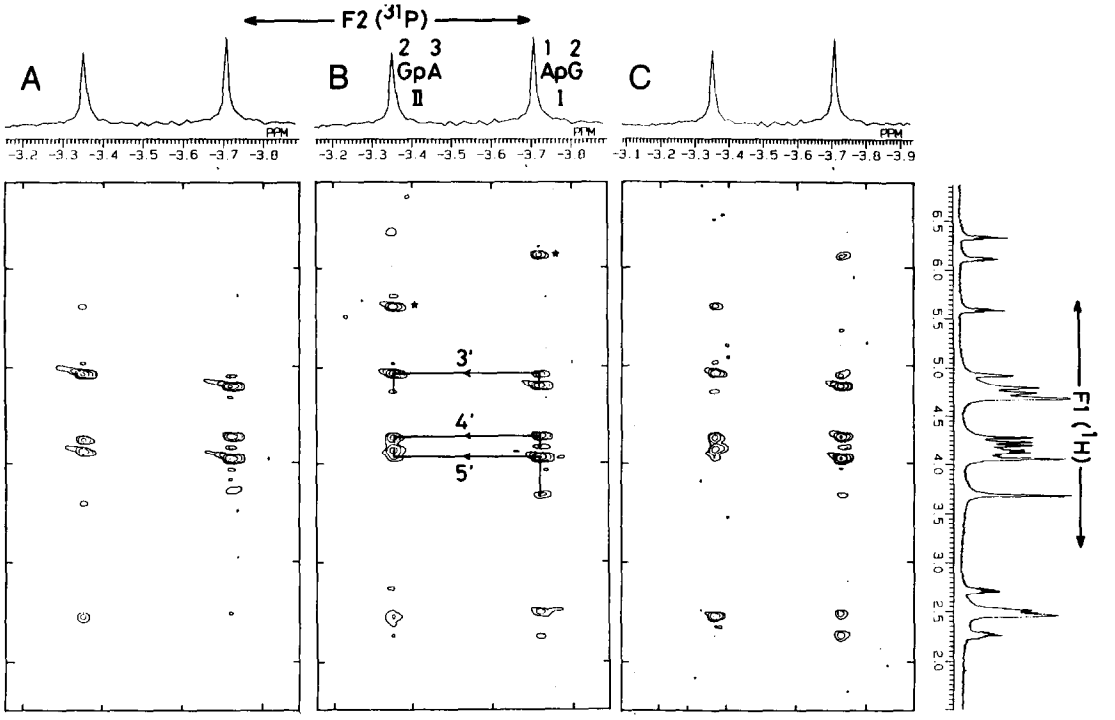

Fig.3. ^{1}H-^{31}P heteronuclear coherence transfer spectra with proton spin locking using MLEV-17 (A,B) and phase-alternated ($x, -x$) pulse cycles (C). Mixing times: 30 ms (A) and 80 ms (B,C). Results of sequential assignments are also indicated in B. Peaks with an asterisk are correlations between the phosphorus and H1'.

also fig.4). Therefore, it is concluded that ribose-a, -b and -c (fig.2) correspond to those of 2G, 1A and 3A, respectively.

In addition, correlation between H1' and the 3'-side phosphate is clearly observed (cross-peaks with an asterisk in fig.3B). Thus, 1AH1' and 2GH1' give relatively strong cross-peaks with P_I and P_{II}, respectively, while 3AH1' (7 bonds distant from P_{II}) gives a very weak peak with P_{II} (fig.3B). This result implies that ^{31}P resonances can be easily assigned if assignments for the H1' resonances are already known. It is particularly useful that ^{31}P resonance assignment can be made according to the data from easily assignable H1' resonances.

The result where the phase-alternated spin lock [7] (fig.1C) is used for the same mixing time (80 ms) is shown in fig.3C for comparison. MLEV-17, assumed to be a more effective spin lock [6], gives stronger correlations between H1' and its proximal phosphorus (5 bonds distant) and better signal-to-noise ratio.

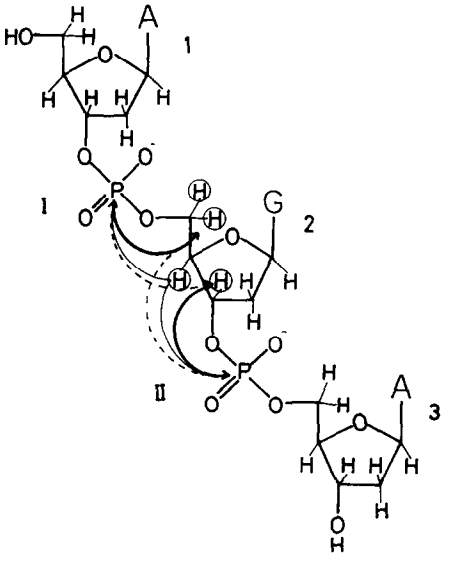

Fig.4. Scheme of sequential correlations for d(ApGpA). The bold, thin and broken lines indicate strong, medium and weak correlations, respectively.

Sequential ^{1}H NMR assignment for oligonucleotides is usually made on the basis of inter-residual NOEs through base protons [11,12]. In this case however, the conformation of the oligonucleotide must be sufficiently rigid for the NOEs to be observed as experienced in a duplex structure. Moreover, the conformation must be regular and standard. The present method using ^{31}P-^{1}H heteronuclear coherence transfer spectroscopy with MLEV-17 spin locking can be used as an alternative when such conditions are not met, as in the cases of stem and loop structures and of any unknown structures.

This method is now being applied to longer oligonucleotides containing a duplex region.

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