Communications to the Editor

A New Approach for Assigning ^{31}P NMR Signals and Correlating Adjacent Nucleotide Deoxyribose Moieties via ^{1}H-Detected Multiple-Quantum NMR. Application to the Adduct of d(TGGT) with the Anticancer Agent (Ethylenediamine)dichloroplatinum

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Compared to ^{1}H, ^{13}C, and ^{15}N NMR spectra, useful ^{31}P spectra of DNA and nucleosomes under physiological conditions can be obtained more readily.[1] Studies of oligodeoxyribonucleotides, where even small ^{31}P shifts can be observed and interpreted[2] and spectra of other nuclei can be observed easily, can provide greater insight into ^{31}P spectral changes on drug, carcinogen, or protein binding to DNA. Unfortunately, full utilization of ^{31}P NMR spectroscopy is hampered by difficulties in signal assignment.[2] The most reliable method to date ($^{17}O/^{18}O$ labeling) is limited to synthetic materials.[3]

We report a new approach for the assignment of the ^{31}P NMR resonances in oligodeoxyribonucleotides via ^{1}H-detected heteronuclear multiple-quantum coherence (HMQC) two-dimensional correlation spectroscopy[4] combined with 2D-NOE methods. This approach exploits the method reported for sensitivity enhancement of heteronuclei in numerous systems;[4] however, we illustrate the advantage of this experiment for information content involving abundant nuclei. The HMQC experiment selects only those ^{1}H nuclei that are spin-coupled to ^{31}P (e.g., deoxyribose H_3',H_5',H_5'') and correlates them with their respective ^{31}P signals. The H_3',H_5',H_5'' signals can be assigned via ^{1}H 2D-NOE methods. Due to the narrow shift range of ^{31}P in these systems, it is essential to record the spectrum as a direct correlation of ^{1}H and ^{31}P chemical shifts by removal of the ^{1}H resonance offset contribution to the F1 dimension.[4a,e] This has been accomplished via the pulse sequence[4e]

$$90°_x(^1H)-\Delta-90°_\phi(^{31}P)-t_1/2-180°_x(^1H)-t_1/2-90°_x(^{31}P)-t_2(^1H,\text{Acq.})$$

The HMQC experiment is superior to conventional two-dimensional heteronuclear correlated spectroscopy,[3b] which suffers from complications associated with the complex spin systems of these fragments. Since the ^{31}P spin is 100% abundant, there will be multiple heteronuclear couplings and extensive homonuclear (^{1}H) couplings, all of which will be of the same order of magnitude, ca. 3-10 Hz. This situation results in significant reduction in enhancement and efficacy in magnetization transfer pulse methods.[4e,5] However, it has been shown that the HMQC method

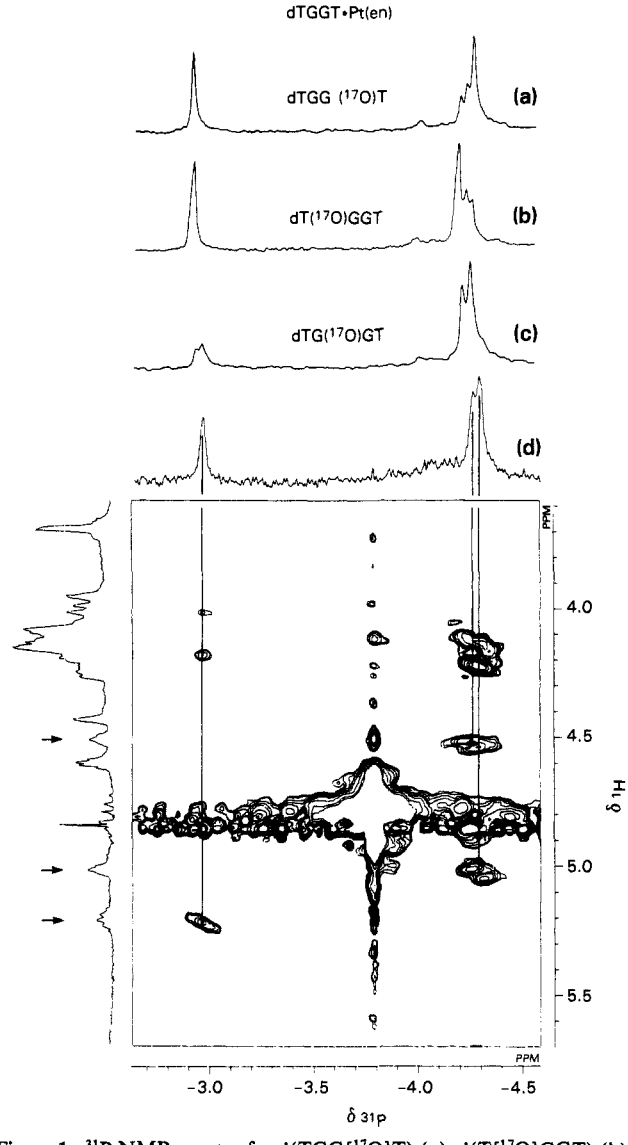

Figure 1. ^{31}P NMR spectra for d(TGG[^{17}O]T) (a), d(T[^{17}O]GGT) (b), and d(TG[^{17}O]GT) (c) adducts with Pt(en)Cl_2, obtained at 81.01 MHz, 15 °C, on an IBM WP200-SY spectrometer. Sample conditions: 5 mM d(TGGT)Pt(en) in D_2O, PIPES 10 buffer (0.01 M Pipes, 0.10 M $NaNO_3$, 0.001 M EDTA), pH 7.0. (d) HMQC contour plot and corresponding ^{31}P and ^{1}H traces obtained on a JEOL GX-400 spectrometer at 20 °C, using a standard 10-mm broad-band probe operated for ^{1}H observe (S/N would be greatly improved by an optimized probe[4f]). Sample conditions: 5 mM d(TGGT)Pt(en) in D_2O, 0.01 M PO_4 buffer, 0.10 M $NaNO_3$, pH* 7.1. Acquisition parameters: 512 × 128 matrix accumulated with Δ-delay = 20 ms, Δt_1 = 1 ms, and total acquisition time ~18 h. Spectral widths were 1000 Hz (F2-^{1}H) and 500 Hz (F1-^{31}P).

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works well for complex, unresolved coupling situations involving metal nuclei.[6] This finding is corroborated in the present study for ^{31}P, wherein good HMQC data are obtained for the complicated spin systems of the DNA backbone (Figure 1). The demonstrated sensitivity advantage of detecting ^{1}H, using an optimized probe,[4f] may become important for the application to larger oligodeoxyribonucleotides.

The alternative relayed single-quantum coherence spectroscopy approach[7] has several disadvantages.[8] First, the efficiency of the relayed method is lower due to the need to optimize two independent transfers to achieve the net correlation, whereas the HMQC method modulates each ^{1}H coherence independent of other ^{1}H–^{31}P couplings. Thus, although the intensity of each correlation peak is dependent on the relative coupling constant, the existence of a correlation is a function of only partial creation of multiple-quantum coherence in each pathway. Second, the assignment of more than one H~P~H correlation is difficult, since ^{31}P chemical shift information is not contained in the relayed COSY-type spectrum. This information is clearly present in the HMQC method, which provides unambiguous correlation of successive deoxyribose moieties for model-independent sequential ^{1}H assignments.[3b]

This approach should be especially important for studying drug–DNA interactions. Because of its effectiveness in the treatment of testicular and ovarian cancers, *cis*-Pt, *cis*-[Pt-$(NH_3)_2Cl_2$], is the most widely used anticancer drug in the US.[9] The molecular target of the drug is most probably DNA.[10] Its DNA adduct, DNA-*cis*-Pt, has an unusual ^{31}P signal (~5% of the signal) at ~−3 ppm,[11] which is 1.2 ppm downfield from the normal signal at −4.2 ppm.[12,13] Related Pt drugs (e.g., (ethylenediamine)dichloroplatinum = Pt(en)Cl_2) also induce this signal but inactive Pt compounds such as *trans*-[$Pt(NH_3)_2Cl_2$] do not.[13] In the same manner, adducts of d(TGGT) with Pt(en)Cl_2 or *cis*-Pt exhibit a downfield signal at ca. −2.9 ppm whereas there are only upfield signals observed for the adduct with *trans*-[$Pt(NH_3)_2Cl_2$].

The adduct of d(TGGT) with Pt(en)Cl_2, d(TGGT)Pt(en), was selected for detailed study by both HMQC/2D-NOE and $^{17}O/^{18}O$ methods. With $^{17}O/^{18}O$ labeling,[14] we can unambiguously assign the ^{31}P signals in the 15 °C spectrum at −2.88, −4.17, and −4.21 ppm to G_PG, T_PG, and G_PT, respectively (Figure 1a–c). Although $^{17}O/^{18}O$ labeling has been applied to actinomycin D binding,[3b,d] to our knowledge, this is the first application to a covalently linked drug adduct.

Using the HMQC approach, each ^{31}P resonance is uniquely correlated with the corresponding H_3' resonance of the preceding nucleotide (Figure 1d). The H_5',H_5'' resonances are resolved for the downfield ^{31}P resonance (−2.96 ppm at 20 °C); however, there is overlap of these resonances for the other two deoxyribose moieties. Improved resolution may be obtained by removing ^{1}H–^{31}P coupling in the F2 dimension;[4e] however, some loss in sensitivity could result due to the additional delay required. In the 2D-NOE study[15] (performed at 5 °C to enhance internucleotide cross relaxation) an NOE cross peak between a T-H_6 (7.58 ppm) and an H_2'' (2.63 ppm) assigned these resonances to T4-H_6 and G3-H_2'', respectively.[16] G3-H_8 (8.96 ppm) was assigned from the cross peak with G3-H_2''; T1-H_6 and G2-H_8 were then assigned by default. The usual treatment[16] led to assignment of all ^{1}H resonances (supplementary material, table) except for T_4-H_5',H_5'', which overlapped with other peaks.

The H_3' signals shifted to 5.23 (G2), 5.04 (G3), 4.62 (T4), and 4.53 ppm (T1) when the temperature was raised to 20 °C. Comparison with the HMQC data (Figure 1) leads to unambiguous assignment of the ^{31}P resonances, which are in full agreement with the $^{17}O/^{18}O$ results. The assignment of the G_PG resonance agrees with conclusions from other methods.[13,17–21]

The d(TGGT)Pt(en) ^{1}H NMR chemical shifts and NOE effects are characteristic of *cis*-Pt adducts in which Pt is bound to N7 of adjacent guanosine bases.[18–20] Good quality spectra are obtained for d(TGGT)Pt(en) even at 5 °C, whereas other systems studied, with at least one flanking 5′ nucleotide, often have several broad signals below 35 °C. The d(TGGT)Pt(en) spectra exhibit a similar temperature dependence; nevertheless, at 5 °C we observe (i) cross peaks between T1-H_6 and T1-H_5',H_5'', indicating a higher degree of rotational freedom for the residue 5′ to G_PGPt, (ii) no internucleotide cross peaks between T1 and G2, indicating that these bases are not stacked, and (iii) "typical" internucleotide cross peaks between G3 and T4, indicating base stacking.

The temperature dependence of d(CGG)-*cis*-Pt ^{31}P spectra was attributed to CG stacking.[19] Above 45 °C, the stacking was eliminated and the observed ^{31}P G_PG shift of ca. −2.9 ppm[19] agrees well with that for d(TGGT)Pt(en) at lower temperature where 5′ T is not stacked. Our study extends the apparent universality of the (G_PG)Pt conformation to lower temperature, to an additional sequence, and, for the first time, to a change in the amine moiety. Since G_PGPt adducts are the major product from the treatment of DNA with Pt anticancer agents,[17] we can infer that adducts similar to d(TGGT)Pt(en) (MW ~2000 daltons) are at least partly responsible for the −3 ppm resonance observed in DNA and nucleosomes (MW ~200 000 daltons).[13]

This report represents the first application of HMQC to ^{31}P NMR and indicates the power of the HMQC/2D-NOE model-independent approach to assign ^{31}P and ^{1}H resonances without resorting to synthetic $^{17}O/^{18}O$ labeling, although the method is confirmed by such labeling. We are planning to extend this new approach to other systems, including larger oligodeoxyribonucleotides, and to evaluate d(TGGT) adducts with other Pt compounds.

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Supplementary Material Available: Entire plot and an expansion of the absorption mode 2D-NOE data and a table of ^{1}H NMR shift assignments for d(TGGT)Pt(en) (3 pages). Ordering information is given on any current masthead page.

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