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Absorption mode two-dimensional NOE spectroscopy of exchangeable protons in oligonucleotides

Vladimír Sklenář*, Bernard R. Brooks+, Gerald Zon° and Ad Bax

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, +Division of Computer Research and Technology, National Institutes of Health, Bethesda, MD 20892 and *°Applied Biosystems, 850 Lincoln Center Dr., Foster City, CA 94404, USA*

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A new NMR method is described for the generation of absorption mode two-dimensional NOE spectra of oligonucleotides in H_2O solution. The method yields spectra that are free of baseline distortions with excellent suppression of the intense H_2O resonance. The method is demonstrated for a sample of the dodecamer d(CGCGAATTCGCG)$_2$. All exchangeable base protons are identified and a number of new types of NOE connectivities are observed.

Oligonucleotide; DNA; 2D NMR; Nuclear Overhauser effect; Water suppression; Hydrogen exchange

1. INTRODUCTION

In recent years, 2D NOE studies of short DNA fragments have gained widespread popularity [1–6]. These studies permit analysis of the solution conformation of DNA. In practice, the vast majority of these studies are conducted in D_2O solution to avoid the dynamic range problems caused by the presence of the H_2O solvent resonance. Therefore, only distances between non-exchangeable protons are usually observed. To obtain more information on the orientation of the bases, it is important to measure the NOEs involving the exchangeable amino and imino protons. Although such studies have been conducted in the past, with few exceptions [7,8] they resulted in low-quality spectra and in all cases required intervention by an experienced operator to minimize the effects of baseline distortion. Here, we demonstrate the use of a new sequence for obtaining absorption mode 2D NOE spectra of oligonucleotides in H_2O solution. A number of NOE interactions, not previously reported, are observed in the spectra recorded with the new experiment.

Correspondence address: V. Sklenář, Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD 20892, USA

* On leave from the Institute of Scientific Instruments, Czechoslovak Academy of Sciences, CS61264 Brno, Czechoslovakia

2. METHODOLOGY

The pulse scheme for the 2D NOE experiment used in the present study is sketched in fig.1a. The first part of this scheme consists of the standard two non-selective pulses separated by the evolution period t_1 [9]. During the mixing period, a strong homogeneity spoiling pulse (duration 30 ms) is applied to defocus all transverse magnetization. Finally, a 1-1 echo sequence [10] is used as a read pulse. As shown elsewhere [10], after EXORCYCLE phase cycling [11] of the $90° - 2\tau - 90°$ refocusing pulse, pure absorptive spectra are obtained with an intensity proportional to $\sin^3(2\pi\delta\tau)$, where δ is the resonance offset of interest in Hz. As shown in fig.1b, it is possible to

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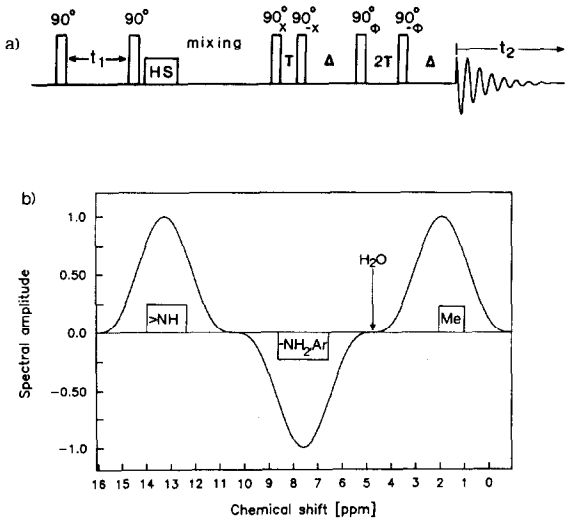

Fig.1. Pulse sequence of the 2D NOE experiment with a 1-1 echo read pulse. The carrier is positioned on the H_2O resonance and the time τ is adjusted to optimize excitation for the region of interest. The phase ϕ is cycled $x,y,-x,-y$, with the acquisition $+,-,+,-$, with all other phases remaining constant. Upon completion of 4 scans, the phases of the first two pulses are changed in the standard manner for obtaining 2D absorptive NOE spectra. The delay Δ is set to a short value 10–500 μs, depending on the line shape. (b) Excitation profile showing a $\sin^3(2\pi\tau\delta)$ dependence, adjusted to maximize excitation in the imino and amino regions of the ^1H spectrum.

choose a single τ value that provides near-optimal excitation of both the imino and amino protons in DNA. Consequently, a single 2D NOE spectrum can show all NOEs to exchangeable protons simultaneously. In the 2D spectrum, all resonances have a $\sin^3(2\pi\delta\tau)$ dependence in the F_2 dimension and do not exhibit any offset dependence in the F_1 dimension. The water suppression per individual scan is on the order of a factor of 50, sufficient to avoid any dynamic range problems. (For probes with a very high Q factor it may be necessary to detune the probe slightly to avoid radiation damping effects during the detection period. For radiation damping, see [12].) The main advantage of the new method is that upon completion of the four-step EXORCYCLE phase cycling very high suppression of the H_2O resonance is obtained and that the 2D spectrum does not require any first or higher order phase corrections. Hence, there is no need for baseline correction routines, a procedure that always presents difficulties in crowded spectra.

3. RESULTS

The sequence of fig.1 is applied to the study of the d(CGCGAATTCGCG)$_2$ duplex ('Dickerson's dodecamer'). Detailed crystallographic data [13] are available for this dodecamer which has been the subject of several NMR studies [14,15]. Fig.2 displays the NOEs to the imino and amino regions of the spectrum. Because of the long duration of the mixing period (100 ms) a substantial number of the cross-peaks observed are due to relayed connectivity (spin diffusion). By comparison of spectra run with different mixing times and at different temperatures it was possible to distinguish direct from relayed NOE connectivities. Comparison with X-ray crystallographic data was used to confirm the distinction between direct and indirect NOE connectivity; all direct connectivities involving inter-base-pair interactions are listed in table 1. Fig.2a shows NOE connectivities between all G1H imino protons to the adjacent C4NH_2 cytidine protons, including the terminal base-pairs. For the A_6T_7 base-pair, the interaction between T3H and A6NH_2 protons is also clearly visible and the A6NH_2 resonances are relatively sharp at 5°C, with the downfield resonance corresponding to the hydrogen-bonded proton. At 35°C (not shown) the exchange between the NH_2 resonances becomes rapid ($\approx$3000 s^{-1}) and only one broad cross-peak to the A6NH_2 resonance is observed. However, it is interesting to note that at 5°C no clear interaction between $(A6NH_2)_5$ and $(T3H)_8$ is observed, possibly due to exchange broadening of the $(A6NH_2)_5$ resonances. The NOE cross-peaks between the G1H and G2NH_2 protons have very low intensity in fig.2 because the NH_2 protons are exchange broadened (exchange rate $\approx$2000 s^{-1}). At 35°C the G2NH_2 resonances have coalesced to relatively narrow lines ($\approx$50 Hz linewidth) and intense NOEs to G1H can be observed for G_2, G_4 and G_{10}. Another weak but interesting direct NOE cross-peak is observed between the $(TH3)_7$ imino proton and the $(T5CH_3)_8$ methyl protons. This peak has an intensity identical to the intra-base T3H-T5CH_3 (r = 4.5 A) connectivities.

Although the direct NOE connectivities are most

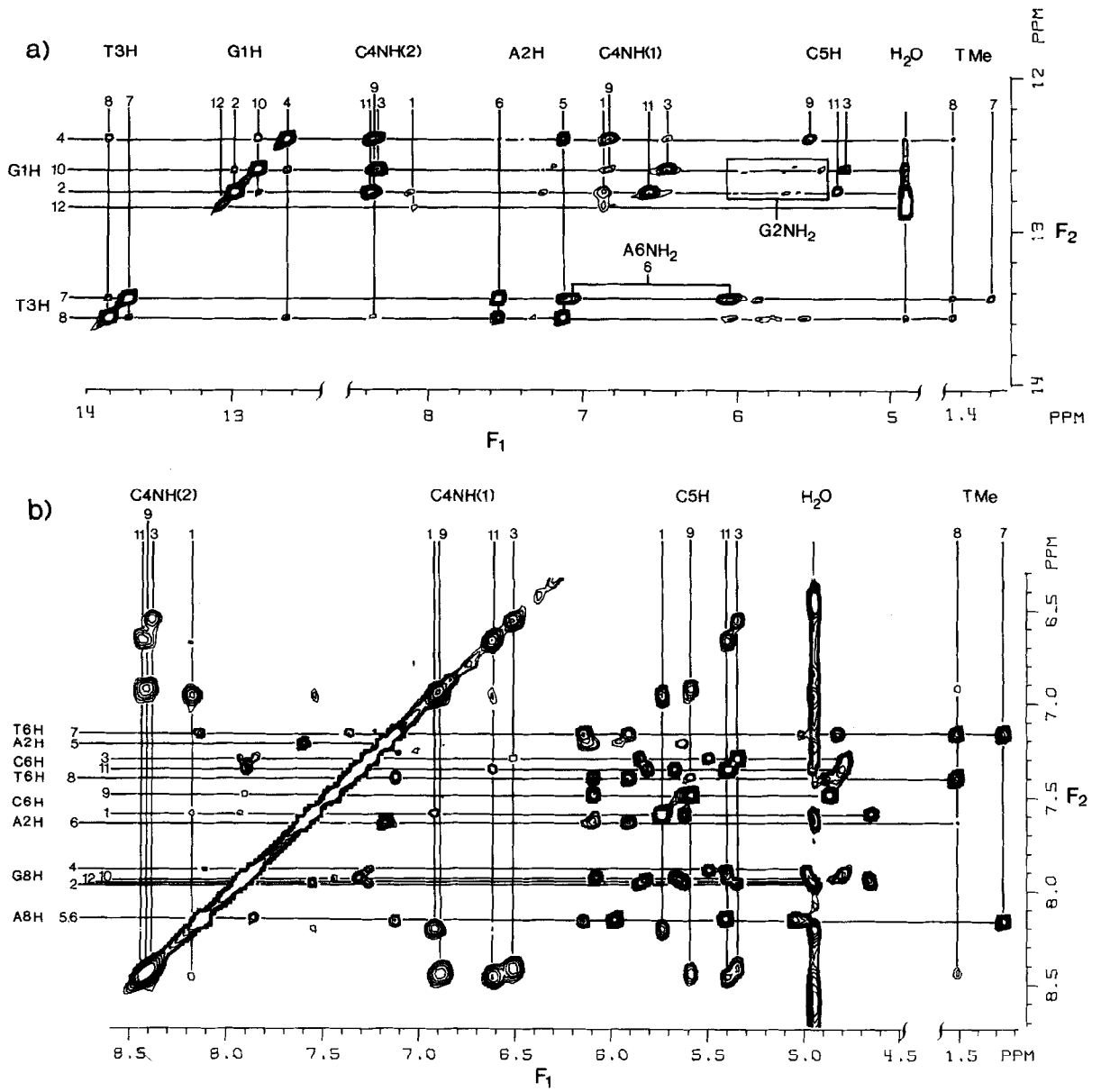

Fig.2. (a) Part of the 2D NOE spectrum displaying the interactions with the imino resonances of d(CGCGAATTCGCG)$_2$ in 90% H_2O/10% D_2O at 5°C, using a 100 ms mixing time. (b) Section of the same spectrum displaying NOEs to the amino and non-exchangeable base protons.

important because of the conformational information they carry, it should be pointed out that relayed connectivity can provide useful assignment information. For example, magnetization relayed from GH1 via an NOE with $C4NH_2$ to C5H (fig.2b) presents a direct means for assignment of GH1 because C5H resonances can be assigned using standard procedures. This particular type of double relay is very efficient because the $C4NH_2$ exhibits relatively rapid 180° flips (on the order of 50 s^{-1} at 5°C) about the C–N bond.

Another interesting feature in the spectrum of

Table 1

Inter-base-pair NOE cross-peaks observed for exchangeable protons in d(CGCGAATTCGCG)$_2$ at 5°C that are not due to spin diffusion

From	To
$(C4NH(2))_1$	$(C4NH(1,2))_{11}$
$(G1H)_2$	$(G1H)_{10}$; $(C4NH(2))_1$
$(G1H)_4$	$(A2H)_5$; $(G1H)_{10}$; $(T3H)_8$; $(C4NH(2))_3$[a]
$(T3H)_7$	$(T5CH_3)_7$; $(T5CH_3)_8$; $(T3H)_8$; $(A2H_5)$[b]
$(T3H)_8$	$(C4NH(2))_9$; $(A2H)_6$
$(C4NH(2))_9$	$(G1H)_{10}$[a]
$(C4NH(1))_9$	$(T5CH_3)_8$

[a] Obscured because of overlap; must be present because relay to C4NH(1) is observed

[b] Overlapping at 5°C but resolved at 35°C

fig.2 is the exchange cross-peak with H_2O, which presents a direct measure for the hydrogen exchange rate [16]. The exchange rate for the imino protons decreases from the ends of the helix towards the center; for the center A_6T_7 base-pair no exchange peak with water is observed, indicating an exchange rate slower than 0.1 s^{-1}.

We have shown that the modified NOE method presented here can be used successfully in the study of oligodeoxyribonucleotides. Since most of the chemical shifts of exchangeable protons in oligoribonucleotides fall in the same regions, the method should also be directly applicable to this class of molecules.

4. EXPERIMENTAL

500 A_{260} units of d(CGCGAATTCGCG)$_2$ were dissolved in 0.5 ml 90% H_2O/10% D_2O, pH 7.0, containing 10 mM phosphate buffer and 100 mM NaCl. The spectrum of which parts are displayed in fig.2 has been recorded with the scheme of fig.1a, with the following parameter settings: t_{mix} = 100 ms, homospoil duration 30 ms, τ = 150 μs, 90° pulse width 36 μs, Δ = 500 μs. The acquisition time in t_2 is 51.2 ms; the acquisition time in t_1 is 35 ms. The total measuring time was 15 h. Digital resolution in the displayed spectrum is 9.76 Hz. 35 Hz exponential line narrowing followed by 34 Hz Gaussian broadening was used in both dimensions. No baseline correction was used.

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