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Application of $^{13}C(\omega_1)$-half-filtered [^{1}H,^{1}H]-NOESY for studies of a complex formed between DNA and a ^{13}C-labeled minor-groove-binding drug

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The complex formed between the anticancer drug 4-[*p*-[*p*-(4-quinolylamino)benzamido]anilino]pyridine (SN 6999) and the decadeoxyribonucleoside nonaphosphate d-(GCATTAATGC)$_2$ was investigated using two-dimensional nuclear Overhauser enhancement spectroscopy (NOESY) with a $^{13}C(\omega_1)$-half-filter. The two quaternary methyl groups in SN 6999 had been labeled with ^{13}C for these experiments. The simplified subspectra of [^{1}H,^{1}H]-NOESY obtained with this procedure greatly facilitate the identification and assignment of intermolecular NOEs. Quite generally, the combined use of isotope labeling and heteronuclear filters in [^{1}H,^{1}H]-NOESY provides an improved experimental basis for structural studies of drug/DNA complexes.

Nuclear magnetic resonance; Drug/oligodeoxyribonucleotide complex; [^{1}H,^{1}H]-NOESY with $^{13}C(\omega_1)$-half-filter; Intermolecular nuclear Overhauser effect

1. INTRODUCTION

^{1}H NMR investigations with biological macromolecules [1] are often limited by incomplete resolution of the signals in homonuclear one-dimensional (1D) or two-dimensional (2D) NMR experiments. Quite generally these limitations become more severe with increasing size of the species studied. A promising avenue toward overcoming these difficulties is heteronuclear editing of the ^{1}H NMR spectra of selectively isotope-labeled molecules [2,3]. In complexes formed by two or several molecules the desired labeling specificity can often readily be achieved by introducing the isotope labels into one of the components before the complex is formed. For structural investigations of such complexes, 2D [^{1}H,^{1}H]-NOESY with heteronuclear half-filters [4–8] is a particularly promising experiment. In this paper a ^{13}C-half-filter experiment is used for studies of a complex formed between an anticancer drug and a synthetic DNA duplex.

The system studied in the present project is the 1:1 complex of d-(GCATTAATGC)$_2$ and the drug SN 6999 (Fig.1), where the latter had been quaternized with a ^{13}C-labeled methyl group at the aza-nitrogens of the quinoline and pyridine moieties (Fig.1a). To single out the protons directly bound to ^{13}C, we used [^{1}H,^{1}H]-NOESY with a $^{13}C(\omega_1)$-half-filter, which discriminates on the basis of the large one-bond heteronuclear spin-spin coupling constant $^1J(^{13}C, ^1H)$ [8]. To illustrate the potentialities of the presently used approach, the results are compared with previous studies of the corresponding, unlabeled system with conventional 2D ^{1}H NMR [9]. To provide a reliable basis for this comparison, the experimental conditions of DNA concentration, buffer, ionic strength, and temperature for the present study were carefully chosen to be identical to those in the earlier experiments with the unlabeled system [9].

2. MATERIALS AND METHODS

The ^{13}C-labeled 4-[*p*-[*p*-(4-quinolylamino)benzamido]anilino]pyridine (SN 6999) (Fig.1a) was prepared by methylation of its free base with 99% ^{13}C enriched CH_3J (Amersham) in *N*, *N*-dimethylacetamide at room temperature, analogous to the preparation of unlabeled SN 6999 [10].

The decanucleotide d-(GCATTAATGC)$_2$ (Fig.1b) was synthesized on an Applied Biosystems 380B DNA-synthesizer using the phosphoamidate method. After deblocking and removing from the solid support, the DNA was purified on a DEAE-Sephacel column (Pharmacia, height 28 cm, diameter 2.5 cm) with a triethylammonium/bicarbonate gradient from 0.3 M to 0.7 M (pH 7.5, total vol. 1.8 litres). After lyophylisation of the decanucleotide-containing fractions, the DNA solution was run over a cation exchange column in the sodium form (Dowex 50W X8, height 50 cm, diameter 1.5 cm, elution with H_2O). The resulting solution was lyophylized and then desalted by gel filtration (Bio Rad P-6DG; column height 50 cm, diameter 1 cm, elution with H_2O). For the NMR measurements in H_2O the

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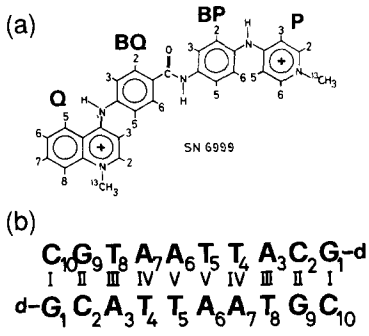

Fig. 1. (a) Structure of 4-[*p*-[*p*-(4-quinolylamino)benzamido]anilino]pyridine (SN 6999). An unambiguous identification of individual nonlabile protons includes the letter for the ring and the ring position, e.g. P2. (b) Structure of the decadeoxyribonucleoside nonaphosphate used in this study. The numeration of the base pairs with roman numerals reflects the 2-fold symmetry observed for the solution conformation of the free duplex [11].

lyophylized decanucleotide was dissolved in 0.5 ml of 50 mM phosphate buffer at pH 7.0, containing 0.1 M NaCl and 1 mM NaN_3. The DNA concentration was 3 mM in duplex. For the NMR spectra taken in ^2H_2O solution this sample was repeatedly lyophilized from 99.8% ^2H_2O, and the final solution was prepared using 99.996% ^2H_2O.

Two-dimensional [^{1}H,^{1}H]-NOESY spectra with a $^{13}C(\omega_1)$-half-filter were recorded on a Bruker AM-500 spectrometer, using the experimental scheme of Fig.2. The acquisition parameters chosen were τ = 6.94 ms, τ_m = 150 ms, t_{1max} = 43 ms, t_{2max} = 229 ms, total recording time about 40 h. Heteronuclear decoupling during t_2 was achieved using WALTZ with suppression of cycling sidebands [12]. Before Fourier transformation the data were multiplied with a cosine-bell in the t_1 dimension, and 3 Hz line-broadening was used along t_2. After Fourier transformation all the rows and columns of the data matrices were baseline-corrected using a third-order polynomial.

3. RESULTS AND DISCUSSION

In studies of intermolecular complexes by delineation of the contacts between the interacting molecules with

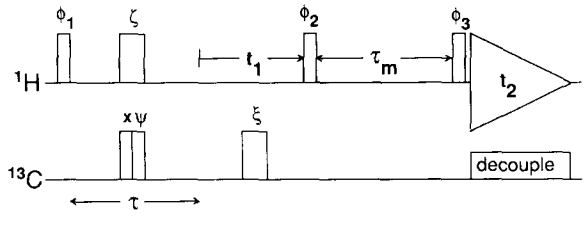

Fig. 2. Experimental scheme for $^{13}C(\omega_1)$-half-filtered [^{1}H,^{1}H]-NOESY with refocussing and heteronuclear broadband decoupling. 90° and 180° pulses are indicated by narrow and wide vertical bars, respectively, t_1 is the evolution period, τ_m the mixing time, t_2 the observation period, and $\tau = 1/^1J(^{13}C,^1H)$ is the filter delay of the half-filter. The following 256-step phase cycling was used: ϕ_1 = [(x, x, y, y)$_{16}$] ($-x$, $-x$, $-y$, $-y$)$_{16}$]$_2$, ζ = [x, x, y, y)$_4$ ($-x$, $-x$, $-y$, $-y$)$_4$]$_8$, ϕ_2 = [(x, x, y, y)$_2$ ($-x$, $-x$, $-y$, $-y$)$_2$]$_{16}$, ϕ_3 = (x, x, y, y, $-x$, $-x$, $-y$, $-y$)$_{32}$, ψ = (x, $-x$)$_{64}$ ($-x$, x)$_{64}$, ξ = [(x)$_{32}$ ($-x$)$_{32}$]$_4$, receiver = (x, x, y, y, $-x$, $-x$, $-y$, $-y$, $-x$, $-x$, $-y$, $-y$, x, x, y, y)$_4$ ($-x$, $-x$, $-y$, $-y$, x, x, y, y, x, x, y, y, $-x$, $-x$, $-y$, $-y$)$_8$ (x, x, y, y, $-x$, $-x$, $-y$, $-y$, $-x$, $-x$, $-y$, $-y$, x, x, y, y)$_4$. The results obtained with ψ = x and ψ = $-x$ were stored in two separate data files. The sum and the difference of these two data sets yielded the *sum spectrum*, and the *difference spectrum*, respectively.

^{1}H-^{1}H NOEs, limitations arise whenever the individual components contain hydrogen atoms with identical chemical shifts. In such situations it may be impossible to distinguish between intramolecular and intermolecular NOEs. In the presently studied system this difficulty arises for the ring methyl protons P-CH_3 and Q-CH_3 in SN 6999 (Fig.1a), for which the chemical shifts coincide closely with those of several deoxyribose protons 4′H, 5′H and 5″H of the DNA [9], and there is the additional difficulty that most of the 4′, 5′H and 5″ protons have not been individually assigned. In contrast, the chemical shifts of the aromatic protons of SN 6999 are unique in the complex with the DNA duplex of Fig.1, so that numerous drug/DNA contact sites could be identified using conventional [^{1}H,^{1}H]-NOESY [9]. Overall, this system is thus already well characterized and the intermolecular ^{1}H-^{1}H NOEs with the *N*-methyl groups of the ligand are therefore particularly suitable for a demonstration of the potentialities of heteronuclear filters for studies of intermolecular interactions [7,13].

In the [^{1}H,^{1}H]-NOESY experiment with $^{13}C(\omega_1)$-half-filter (Fig.2), the difference spectrum (Fig.3b) contains the diagonal peaks from the two ^{13}C-labeled methyl groups and from protons bound to ^{13}C in natural abundance, and all the cross-peaks between ^{13}C-bound protons in ω_1 and all other protons in ω_2. The sum spectrum (Fig.3a) contains all the other diagonal peaks and cross-peaks, which would be present also in the conventional [^{1}H,^{1}H]-NOESY experiment. Fig.4a shows the cross-section along ω_2 through the sum spectrum of Fig.3a taken at ω_1 = 4.10 ppm, which is the chemical shift of the P-$^{13}CH_3$ resonance. The cross-section includes all the cross-peaks with 4′, 5′ and 5″ proton resonances of the decanucleotide which have similar chemical shifts as the P-$^{13}CH_3$ protons of SN 6999. In a conventional [^{1}H,^{1}H]-NOESY spectrum of the complex, the overlap of the P-$^{13}CH_3$ proton resonance with these 4′, 5′ and 5″ proton lines would thus prevent the unambiguous distinction between intermolecular ligand/DNA NOEs and intramolecular NOEs between different protons of the DNA. The comparison of this cross-section with the corresponding cross-section taken through the diagonal peak of the P-$^{13}CH_3$ resonance in the difference spectrum (Fig.4b) demonstrates the advantages gained by subspectral editing with the use of the $^{113}C(\omega_1)$-half-filter. This cross-section contains exclusively NOEs with the ^{13}C-bound protons of the P-$^{13}CH_3$ group, which includes both intramolecular interactions with other protons of SN 6999 and intermolecular interactions with protons of the DNA. Intermolecular NOEs with the P-$^{13}CH_3$ protons are observed for $A_3$2H, $A_7$2A, and $T_4$1′H.

Similar to the situation with P-$^{13}CH_3$, the Q-$^{13}CH_3$ proton resonance overlaps with several of the 4′H, 5′H and 5″H lines of the DNA. Fig.4c displays a cross-section along ω_2 through the sum spectrum (Fig.3a)

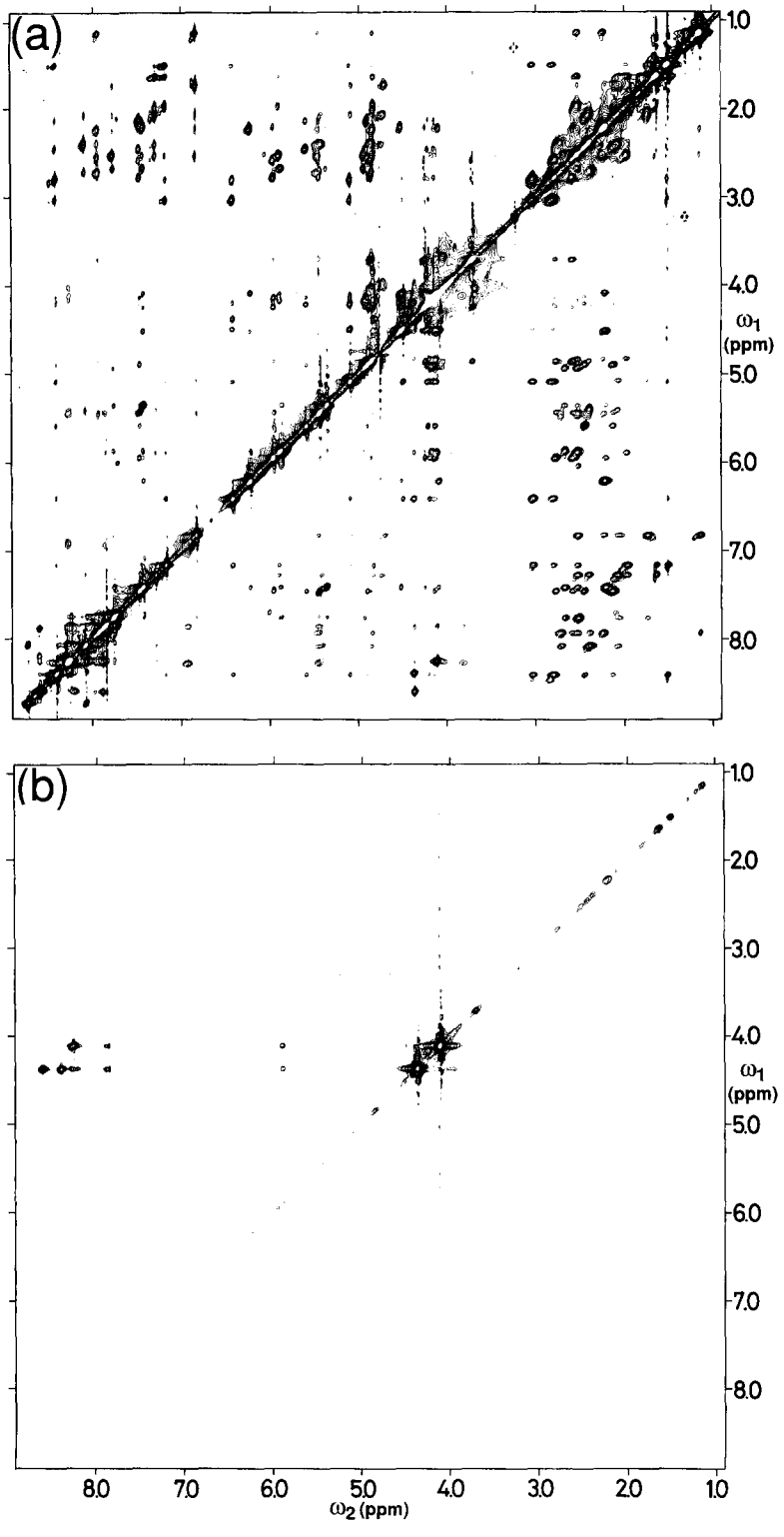

Fig.3. [^{1}H,^{1}H]-NOESY spectra recorded with a ^{13}C(ω_1)-half-filter (Fig.2) of the 1:1 complex formed between d-(GCATTAATGC)$_2$ and the drug SN 6999 with ^{13}C-labeled P-CH$_3$ and Q-CH$_3$ groups (Fig.1). (DNA duplex concentration 3 mM, solvent ^{2}H$_2$O, 0.05 M phosphate buffer p^{2}H = 7.0, 0.1 M NaCl, 1 mM NaN$_3$, T = 301 K, ^{1}H frequency 500 MHz.) (a) Sum spectrum. (b) Difference spectrum.

taken at ω_1 = 4.36 ppm, which is the chemical shift of the Q-^{13}CH$_3$ resonance. Again, numerous intramolecular NOEs between different protons of the DNA can be seen in this trace. In the corresponding cross-section of the difference spectrum (Fig.4d) several intermolecular NOESY cross-peaks involving Q-^{13}CH$_3$

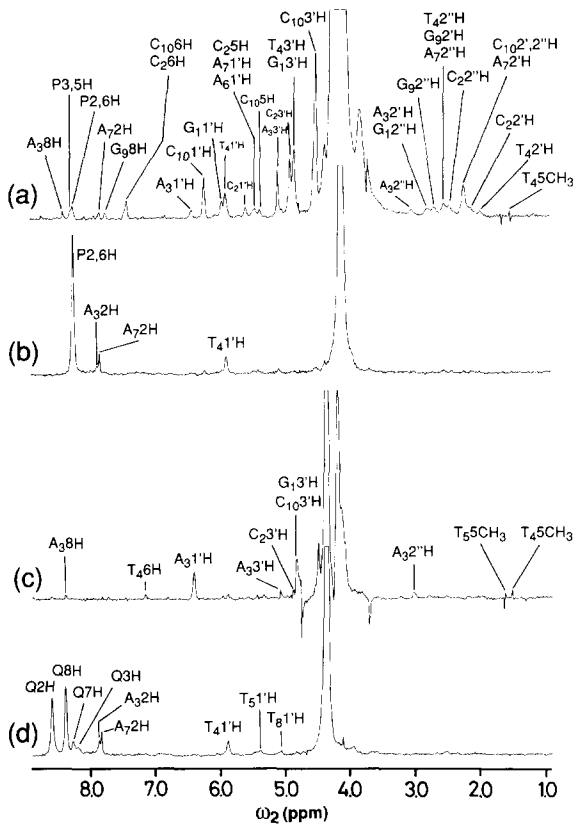

Fig.4. Cross-sections along ω_2 from the NOESY spectra of Fig.3. (a) and (b) were taken at ω_1 = 4.10 ppm, which is the chemical shift of P-$^{13}CH_3$, where (a) is from the sum spectrum, and (b) from the difference spectrum. (c) and (d) were taken at ω_1(Q-$^{13}CH_3$) = 4.36 ppm, where (c) is from the sum spectrum, and (d) from the difference spectrum. Individual cross-peaks are identified with the protons of the DNA (see Fig.1b for the numeration of the nucleotides used) that give intermolecular NOEs with P-$^{13}CH_3$, or Q-$^{13}CH_3$, respectively, or with the protons of the ligand that give rise to intramolecular NOEs (see Fig.1a for the notation used).

and protons of the DNA can be detected, namely between Q-$^{13}CH_3$ and $A_3$2H, $A_7$2H, $T_4$1'H, $T_5$1'H, $T_8$1'H.

The intermolecular proton-proton contacts between P-$^{13}CH_3$ and Q-$^{13}CH_3$ of SN 6999 and the decanucleotide observed in the difference spectrum from the [^{1}H,^{1}H]-NOESY experiment with $^{13}C(\omega_1)$-half-filter corroborate the following conclusions drawn from [^{1}H,^{1}H]-NOESY experiments with the unlabeled system [9]: (i) the methyl protons P-$^{13}CH_3$ and Q-$^{13}CH_3$ are close to protons accessible in the minor groove of the DNA; (ii) all intermolecular contacts found here are consistent with the previous proposal that SN 6999 binds only within the AT-stretch of d-(GCATTAATGC)$_2$. The different number of intermolecular [^{1}H,^{1}H]-NOESY cross-peaks with P-$^{13}CH_3$ and Q-$^{13}CH_3$ protons identified in the spectra of Fig.4b and d, and in earlier work [9], using data from conventional [^{1}H, ^{1}H]-NOESY spectra recorded under otherwise identical conditions are due to the limitations of earlier experiments by the chemical shift degeneracies between P-$^{13}CH_3$ and Q-$^{13}CH_3$ protons of SN 6999 and 4'H, 5'H and 5"H of the DNA. Thus the present study emphasizes the usefulness of [^{1}H,^{1}H]-NOESY spectra recorded with X-half-filters [8] as an aid in identifying intermolecular proton-proton contacts in DNA/drug complexes. Clearly, the use of this approach can be extended to DNA complexes with other classes of ligands, eventually also including other macromolecules, such as proteins.

In principle, the information contained in the cross-sections of Fig.4b and d could be obtained also from a [^{13}C,^{1}H]-COSY experiment with ^{1}H-^{1}H NOE-relay [14]. However, the ω_1-frequency axis in heteronuclear COSY with NOE-relay contains ^{13}C chemical shifts rather than ^{1}H chemical shifts. As a consequence, this experiment does not provide the information from the sum spectrum of the [^{1}H,^{1}H]-NMR experiment with $^{13}C(\omega_1)$-half-filter (Fig.4a and c). In the present study the simultaneous availability of the sum spectrum *and* the difference spectrum proved to be of great help for the individual assignments of the cross-peaks in the difference spectrum. Since the sensitivity of [^{1}H,^{1}H]-NOESY with $^{13}C(\omega_1)$-half-filter is comparable to that of [^{13}C,^{1}H]-COSY with ^{1}H-^{1}H NOE-relay, the presently used half-filter technique [8] should be superior to the NOE relay experiment [14] in practical studies of intermolecular contacts.

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REFERENCES

[1] Wüthrich, K. (1986) NMR of Proteins and Nucleic Acids, Wiley, New York.
[2] Le Master, D.M. and Richards, F.M. (1985) Biochemistry 24, 7263-7268.
[3] Griffey, R.H. and Redfield, A.G. (1987) Q. Rev. Biophys. 19, 51-82.
[4] Bolton, P.H. (1985) J. Magn. Reson. 62, 143-146.
[5] Otting, G., Senn, H., Wagner, G. and Wüthrich, K. (1986) J. Magn. Reson. 70, 500-505.
[6] Fesik, S.W., Gampe, R.T. Jr and Rockway, T.W. (1987) J. Magn. Reson. 74, 366-371.
[7] Fesik, S.W., Luly, J.R., Erickson, J.W. and Abad-Zapatero, C. (1988) Biochemistry 27, 8297-8301.
[8] Otting, G. and Wüthrich, K. (1990) Q. Rev. Biophys., in press.
[9] Leupin, W., Chazin, W.J., Hyberts, S., Denny, W.A. and Wüthrich, K. (1986) Biochemistry 25, 5902-5910.
[10] Cain, B.F., Atwell, G.J. and Seelye, R.N. (1969) J. Med. Chem. 12, 199-206.
[11] Chazin, W.J., Wüthrich, K., Hyberts, S., Rance, M., Denny, W.A. and Leupin, W. (1986) J. Mol. Biol. 190, 439-453.
[12] Shaka, A.J., Barker, P.B., Bauer, C.J. and Freeman, R. (1986) J. Magn. Reson. 67, 396-401.
[13] Senn, H., Otting, G. and Wüthrich, K. (1987) J. Am. Chem. Soc. 109, 1090-1092.
[14] Rance, M., Wright, P.E., Messerle, B.A. and Field, L.D. (1987) J. Am. Chem. Soc. 109, 1591-1593.