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^{1}H NMR study of the interaction of bacteriophage λ Cro protein with the O_{R3} operator

II. Assignment of the non-exchangeable proton resonances of the O_{R3} operator

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Abstract. The 17 base pair operator O_{R3} oligonucleotide, which is the preferential binding site for the Cro repressor of phage λ, was studied by two-dimensional NMR spectroscopy. A sequential assignment procedure based on two-dimensional Nuclear Overhauser Effect (NOESY) and scalar coupling correlated (COSY) NMR spectroscopy, together with the knowledge of the oligodesoxynucleotide sequence, made it possible to assign the non-exchangeable base protons and the H1′ and the H2′–H2″ sugar protons of the O_{R3} operator DNA. The pattern of the observed NOE connectivities is consistent with a right-handed helical DNA structure. The base and sugar proton assignments provide the necessary information for further studies of the O_{R3} operator – Cro repressor interaction.

Key words: O_{R3} operator, nuclear magnetic resonance, assignment of proton resonances, two-dimensional nuclear Overhauser effect spectroscopy

Introduction

The key components of the genetic switch regulating the lytic and lysogenic life mode of the bacteriophage λ are Cro, the *cI* repressor and their sequence specific binding sites on the operators regions of the phage genome. Cro is one of the most thouroughly investigated DNA binding proteins and, because of its relatively small size, is particularly suitable for high resolution NMR studies. Since there is considerable evidence for a high degree of structural similarity in the DNA binding domains of gene regulatory proteins from x-ray diffraction studies of Cro (Anderson et al. 1981), the catabolite gene activator protein (McKay and Steitz 1981) and the DNA binding domain of the *cI* repressor (Pabo and Lewis 1982), and from the predicted secondary structures (Ptitsyn et al. 1982) and comparison of the amino acid sequences of many other DNA binding proteins (Matthews et al. 1982; Sauer et al. 1982; Takeda et al. 1983), studies of the interaction of Cro with phage λ operator DNA may lead toward a better understanding of the general pattern of protein-DNA recognition.

In a previous NMR investigation we studied the shifts of the imino proton resonance positions of the O_{R3} operator, the preferential binding site for Cro on the phage λ genome, upon complex formation with Cro and showed that the specific binding leads to changes in the local helix geometry while stabilizing the overall double helical structure of the DNA (Kirpichnikov et al. 1984). The changes of the operator structure may be of general importance for the specific interaction of repressors with DNA. Even though the imino proton resonances, because of the high sensitivity of their chemical shift values to changes in the local geometry of the helix (Hilbers 1979), can serve as a convenient probe for the detection of changes in the helical structure, they do not provide information on the contact sites between the protein and the DNA. More likely candidates for a direct contact with the protein are the accessible protons on the outside of the DNA helix (Ohlendorf et al. 1982). It should be possible to identify the protons involved in a direct contact by the detection of an intermolecular NOE. The first step toward this goal has to be the resonance assignment of the non-exchangeable DNA protons.

Only very recently have sequential assignment strategies for the non-exchangeable protons of oligonucleotides been proposed (Feigon et al. 1983; Hare et al. 1983; Scheek et al. 1983, 1984; Weiss et al. 1984a; Clore and Gronenborn 1983, 1984; Buck et al.

Abbreviations: COSY: correlated spectroscopy; FID: free induction decay; NOE: nuclear Overhauser effect; NOESY: nuclear Overhauser effect spectroscopy; RD: relaxation delay; TSP: sodium 3-trimethylsilyl-(2,2,3,3-$^2H_4$)propionate; EDTA: sodium ethylendiamine tetraacetate

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1984). These strategies are mostly based on two-dimensional ^{1}H NMR techniques and make it possible to assign the non-exchangeable base and sugar proton resonances for DNA duplexes of small size (up to about 25 base pairs). Since it is known from CD studies (Kurochkin et al. 1984) that the O_{R3} operator adopts a right-handed *B*-helical conformation in solution, a certain network of spin connectivities can be expected, which allows the sequential assignment of the non-exchangeable proton resonances. In the present paper we report the assignment of the base and the H1′, H2′ and H2″ sugar proton resonances of O_{R3}. These assignments are the prerequisite for the further investigation of the specific O_{R3}-Cro complex.

Materials and methods

The 17 base pair oligodeoxynucleotide representing the λ O_{R3} operator site was synthesized and prepared for NMR work as reported previously (Kirpichnikov et al. 1984; Kurochkin et al. 1984). For the two-dimensional NMR experiments, the 17 base pair duplex strand was dissolved in 0.2 M NaCl, 5×10^{-4} M EDTA, 0.02% NaN_3. The pH of the solution was 7.57. The DNA solution was repeatedly dried and the powder redissolved in 99.95% and, finally, in 99.996% D_2O. The final concentration of DNA duplex was 45 mg/mL (about 4 mM in dimer), assuming an optical density at 260 nm of 20 cm^{-1} for a 1 mg/mL solution.

The NMR experiments were carried out at 500 MHz on a Bruker AM 500 spectrometer, equipped with an Aspect 3000 computer. Two-dimensional nuclear Overhauser effect spectra (NOESY) were recorded by using the pulse sequence (RD – 90° – t_1 – 90° – τ_m – 90° – observation pulse)$_n$ as described (Macura et al. 1981; Wider et al. 1984). The delay, t_1, is the evolution period, τ_m marks a mixing time during which exchange of longitudinal magnetization occurs, and RD is a relaxation delay. Three mixing times (100, 200, 350 ms) were used to account for the different buildup rates of the Nuclear Overhauser Effect for different groups of protons in the molecule. Either 128 or 256 free induction decays (FID's), 4096 data points each, were recorded over a 4450 Hz sweep width in the first dimension for each spectrum. Before Fourier transformation the FID's were weighted with a 10°–45° shifted square sine-bell function (Wider et al. 1984) in the first dimension and an unshifted sine-bell function in the second dimension. In order to achieve the same resolution in both dimensions for symmetrization of the spectra, the interferograms, obtained after the first Fourier transformation, were zero-filled to 2048 points before their transformation.

The two-dimensional homonuclear correlated spectroscopy experiment (COSY) was carried out as described (Aue et al. 1976), using the pulse sequence (RD – 90° – t_1 – 45° – observation pulse)$_n$ (Bax and Freeman 1981). A total of 128 FID's was recorded, each of 2048 data points, with the carrier frequency at the centre of the spectrum. The FID's in both dimensions were weighted with a squared sine-bell function before zero-filling and Fourier transformation.

To eliminated artefacts and strong axial peaks and to achieve quadrature detection along the F_2 and F_1 axis of the 2-*D* spectrum, a 32-step phase cycling routine, as proposed by Gurevich et al. (1984), was used for all measurements. All two-dimensional spectra are presented as contour plots in the absolute-value mode. Chemical shifts are expressed relative to external TSP; the temperature in all experiments was 25 °C.

Results

O_{R3} is an asymmetric duplex with the sequence

5′ *d* $T_1A_2T_3C_4A_5C_6C_7G_8C_9A_{10}A_{11}G_{12}G_{13}G_{14}A_{15}T_{16}A_{17}$ 3′
3′ *d* $A_1T_2A_3G_4T_5G_6G_7C_8G_9T_{10}T_{11}C_{12}C_{13}C_{14}T_{15}A_{16}T_{17}$ 5′

For convenience, all bases in both strands are numbered from left to right; i.e., T_1 is the first (5′) base of the upper strand, A_1 is the last (3′) base of the lower strand.

Classification according to resonance type

Out of a total of 387 protons from all 17 base pairs, 316 protons do not exchange with water under normal conditions and therefore give rise to a NMR signal in D_2O. The latter include the aromatic H-8 and H-2 protons of adenine, the H-8 proton of guanine, the H-6 and H-5 protons of cytidine, and the H-6 proton of thymidine as well as the thymidin methyl group and all sugar backbone protons. Figure 1 shows the 500 MHz ^{1}H NMR spectrum of the duplex 17mer in 99.996% D_2O at 25 °C. A comparison of this spectrum with the spectra of smaller oligonucleotides already published (Feigon et al. 1983; Clore and Gronenborn 1984) leads to a first classification as to what general proton types the signals belong.

The limited chemical shift dispersion among the sugar H-5′, H-5″, H-4′, and H-3′ proton resonances in combination with the inherently broad signals encountered in absolute value representations precludes a thorough assignment for these resonances at the present time. This leaves 180 proton resonances to be assigned, namely the base H-8, H-2, and H-6 proton resonances between 8.5 and 7.0 ppm, the cytidine H-5

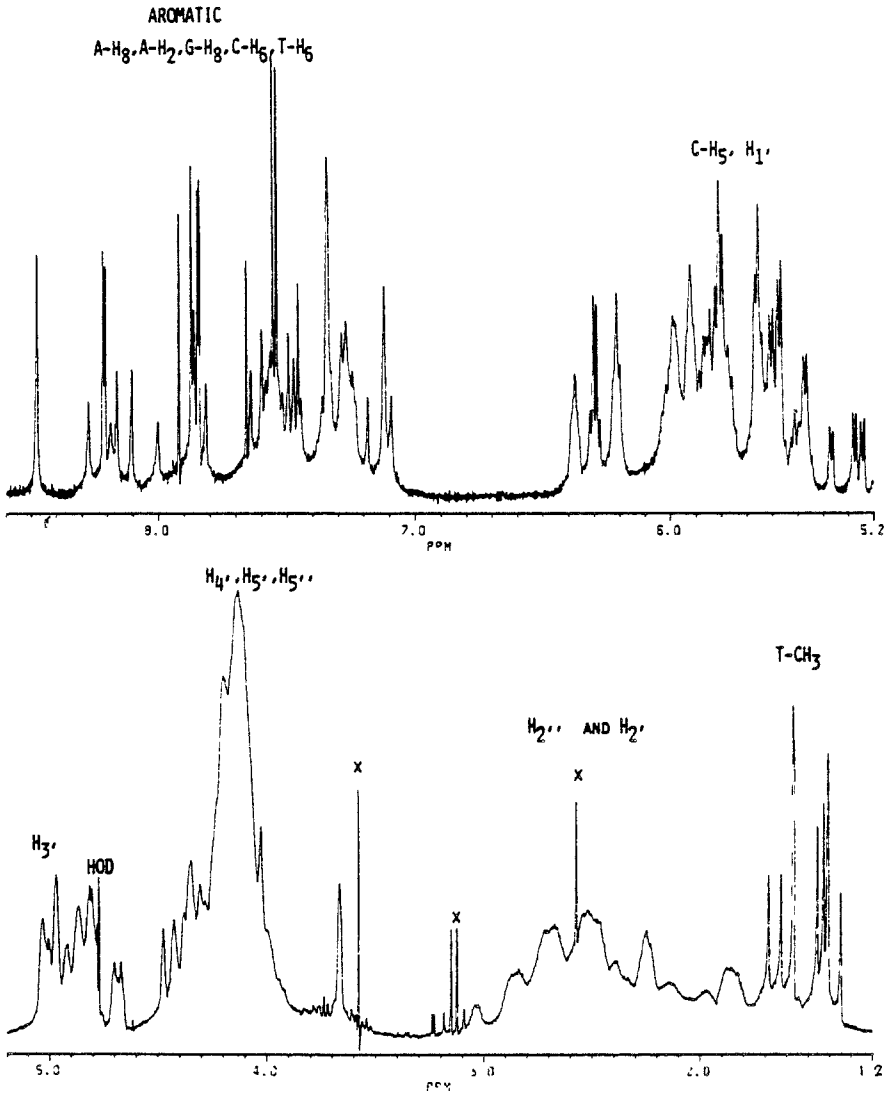

Fig. 1. Resolution enhanced 500 MHz ^{1}H NMR spectrum of O_{R3} operator in D_2O at 25 °C. Classifications according to the general proton type are given above the appropriate spectral regions. The peaks marked × arise from buffer substances and from low-Molecular-mass impurities

and sugar H-1′ proton resonances between 6.5 and 5.2 ppm, the sugar H-2′ and H-2″ proton resonances between 3.1 and 1.7 ppm, and finally the thymidine methyl proton resonances between 1.7 and 1.3 ppm. Within each region, further distinctions can be made. The H-2′ and H-2″ resonances of the purine residues are generally found downfield from those of the pyrimidine residues (Feigon et al. 1983; Clore and Gronenborn 1984; Tran-Dinh et al. 1983). Furthermore, for single-stranded DNA it is noted that the H-2′, H-2″ resonances of the internal pyrimidine residues are well separated, whereas those of the internal purine residues have very similar chemical shifts (Tran-Dinh et al. 1983). In the H-1′/H-5 region the two types of resonances are easily distinguished on account of their multiplet structure. The H-5 protons are doublets due to their coupling with the H-6 protons of their own residue, whereas the H-1′ resonances are triplets due to coupling between the H-1′ proton and the H-2″ protons. Thus, in Fig. 2A, the resonances from six of the eight C residues can easily be recognized between 5.2 and 5.7 ppm. Finally, in the aromatic part of the spectrum, the adenine H-2 proton resonances can be identified on account of their long spin-relaxation times in D_2O (i.e. narrow line widths). An inversion-recovery pulse sequence ($180° - \tau - 90°$) can be used to differentiate between H-2 and H-8 or H-6 resonances (Weiss et al. 1984b). From Fig. 2B the position of all 9 adenine H-2 proton resonances is obvious.

Identification of coupled spin systems

After the classification of the signals according to their general type the next step was the identification of those signals which belong to the same residue by a two-dimensional homonuclear correlated spectro-

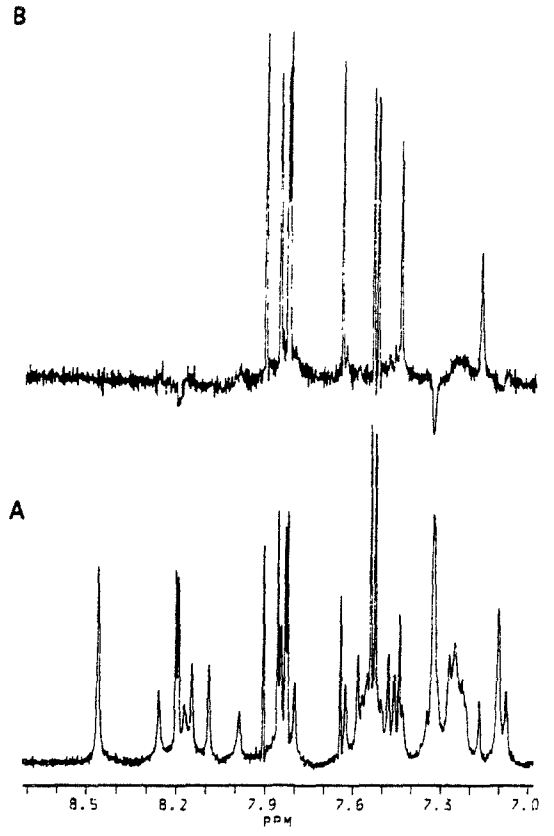

Fig. 2A and B. Identification of the nine adenine H-2 proton resonances. **A** The aromatic region of O_{R3} operator at 25°C. **B** Inversion-recovery spectrum (180° – τ – 90°) identifies the nine adenine H-2 resonances at 25°C. The waiting time between the first and the second pulse of the cycle was 1.2 s. To facilitate a comparison between the spectra, the spectrum in **B** is 180° phase-shifted

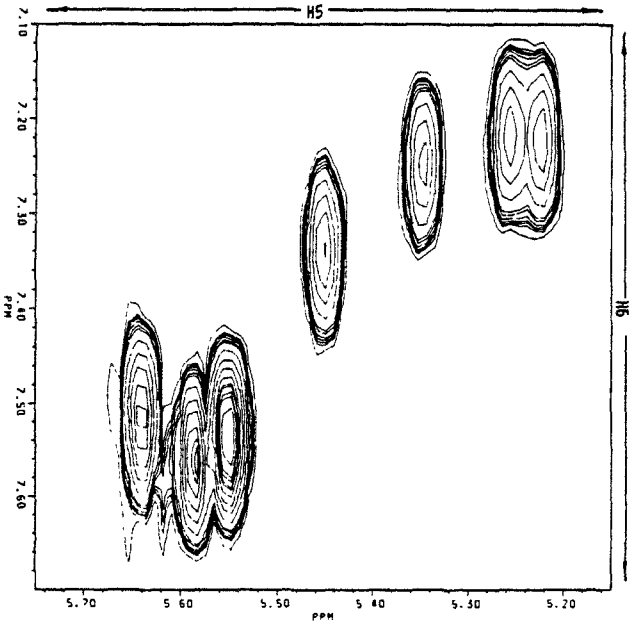

Fig. 3. Portion of an unsymmetrized COSY spectrum showing the spin-coupled H-6/H-5 proton pairs of the eight cytidine residues

scopy experiment (COSY). In a COSY spectrum, the off-diagonal cross peaks are between homonuclear J-coupled proton resonances. The protons in DNA that are strongly J-coupled include the sugar protons (e.g. H-1′–H-2″, H-1′–H-2′) and the H-5 and H-6 protons of cytidine. Figure 3 shows a portion of a 2-*D* COSY spectrum where the cross-peaks between cytidine H-5 and H-6 are expected to appear. All eight connectivities are individually resolved, thus allowing a direct identification of all eight pairs of cytidine H-5 and H-6 protons.

Further information available from the COSY experiment is the interconnection of the H-1′ and H-2′ and H-2″ protons of the same nucleotidyl unit. These connectivities can be used to check the assignment of resonances found in the NOESY experiment.

Sequential resonance assignment

The next and most important step was to establish the interconnectivities between base protons and sugar protons of the same nucleotidyl unit and of the adjacent subunits. This can be done by means of a 2-*D* NOESY experiment. In such an experiment, dipole-dipole cross-relaxation between a pair of closely spaced protons results in the appearance of an off-diagonal peak linking the two corresponding diagonal resonances, thereby demonstrating the proximity of the two protons (Macura et al. 1981). The efficiency of the cross-saturation is proportional to the inverse of the sixth power of the distance (Noggle and Schirmer 1971). Therefore, in the absence of second-order NOE's, only protons which are less than ~0.4 nm apart give rise to a pair of off-diagonal peaks in the spectrum.

Quite recently a number of NOE sequential resonance assignment strategies for oligonucleotides, based on the known structures of right-handed DNA, have been extensively discussed (Feigon et al. 1983; Hare et al. 1983; Scheek et al. 1983, 1984; Weiss et al. 1984a; Clore and Gronenborn 1983, 1984; Buck et al. 1984). In short, they state that each purine H-8 or pyrimidine H-6 proton will be close in space to its own deoxyribose H-1′, H-2′, and H-2″ protons as well as to the H-1′, H-2′, and H-2″ protons of its 5′ neighbour along the same strand. These distances are short enough to generate NOE cross-peaks whereas the distances to the corresponding protons of the 3′ flanking nucleotidyl unit are such that no NOE's are observed. Thus a cross-relaxation network involving this set of eight protons extends throughout each strand of the DNA duplex. In addition, a second cross-relaxation network exists whenever a pyrimidine residue occurs in the strand. There, the cytidine H-5 or thymidine CH_3 resonances show strong intra-

nucleotide NOE's with their own H-6 resonances but also inter-nucleotide NOE's with the base, H-2′, and H-2″ resonances of their adjacent 5′ neighbour.

Except for the adenine H-2 protons, which, together with the exchangeable imino and amino protons, are part of a separate cross-relaxation network (Weiss et al. 1984b), these two sets of NOE connectivities should suffice to assign all resonances from both strands. In principle, the DNA sequence being known, one can "walk" from base to base along one strand via their intra- and inter-base NOE connectivities. In practice however, the limited chemical shift dispersion may lead to discontinuities in the assignment ladder. It is helpful then to have independant starting points to continue the assignment procedure. Such unique starting points are, for example, all 5′ *X-T* or 5′ *X-C* steps (*X* denotes any of the four bases) because of the strong inter-residue NOE between the base H-6/H-8 proton and the thymidine methyl group or the cytidine H-5 proton.

In the O_{R^3} sequence, there are four 5′ *A-T* steps, one 5′ *G-T* step, one 5′ *C-T* step, and one 5′ *T-T* step, all of them occuring in the lower strand. The rightmost part of Fig. 4 shows the region of the 2-*D* NOESY spectrum, where the cross-peaks between base protons and the thymidine methyl group are to be expected. The two unique cross-peaks between G_6 H-8/T_5 CH_3 at 1.35 ppm and C_{12} H-6/T_{11} CH_3 at 1.63 ppm are easily recognized as is the cross-peak between T_{11} H-6 and T_{10} CH_3 at 1.68 ppm. Seven out of nine resolved thymidine methyl resonances can thus be labeled as belonging to a 5′ *X-T* step, which identifies the last two as belonging to the two 5′ terminal *T's* from both strands (T_1 and T_{17}). This agrees well with the absence of any inter-residue cross-peaks in the H-6/H-1′ region (Fig. 4 left part).

To illustrate the sequential assignment routine, Fig. 4 shows an example for the sequences 5′ $T_1A_2T_3C_4A_5$ in the upper strand and 5′ $T_{17}A_{16}T_{15}C_{14}C_{13}$ in the lower strand. From the connectivities between the base protons and the thymidine methyl groups, the signal at 7.345 ppm was identified as belonging to the H-6 protons of T_1 and T_{17}. This signal has only one cross-peak to the H-1′ region at

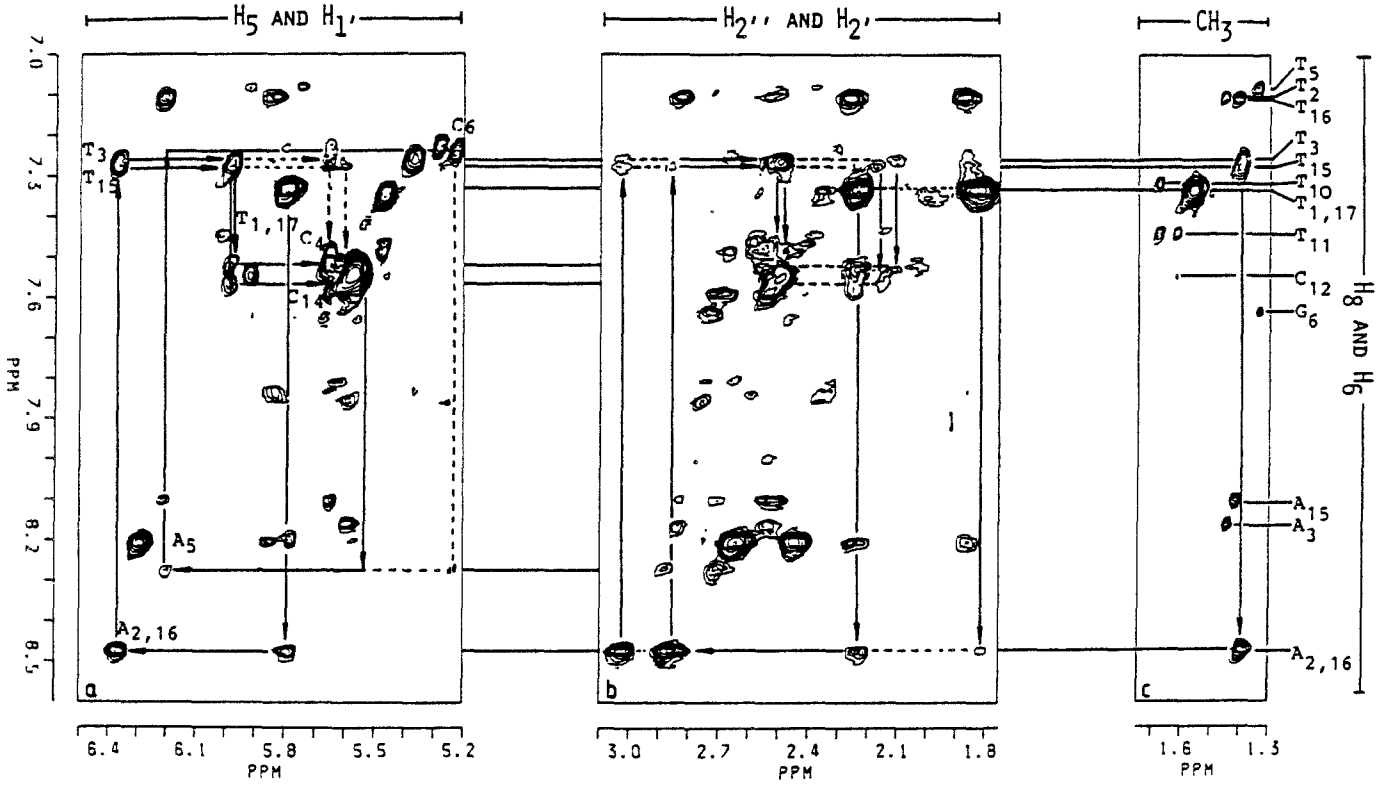

Fig. 4a–c. Portion of a symmetrized NOESY spectrum (350 ms mixing time) to illustrate the sequential assignment procedure. The NOE connectivities relating the bases 5′ $T_1A_2T_3C_4A_5$ in the upper strand and 5′ $T_{17}A_{16}T_{15}C_{14}C_{13}$ in the lower strand are indicated (for further explanation see text). **a** illustrates the role of the H-5 and H-1′ protons. Solid lines indicate NOE connectivities involving the base protons, their own H-1′ protons, and their 5′ neighbouring H-1′ protons. Broken lines mark NOE connectivities between base protons and H-5 protons of a 3′ neighbouring cytidine residue. **b** shows the cross-relaxation pattern among base protons and H-2″ and H-2′ protons. Broken lines connect the H-2″/H-2′ proton pair of one nucleotidyl unit, solid lines mark NOE connectivities. **c** shows all eighteen cross-peaks which involve a thymidine methyl group. Seven result from a 5′ *X-T* step in the sequence as cross-peak between the aromatic proton of base *X* and the methyl group of a 3′ adjacent thymidine residue. They are useful starting points for the sequential assignment procedure

5.79 ppm. Two adenine H-8 signals at 8.476 ppm also show a cross-peak to the same H-1′ resonance and to another H-1′ resonance at 6.37 ppm. Therefore the signal at 8.476 ppm belongs to H-8 of adenines 2 and 16, the signal at 6.37 ppm belongs to H-1′ of adenines 2 and 16, and the signal at 5.79 ppm belongs to the 5′ neighbouring thymidines 1 and 17. This finding is further substantiated by the NOE connectivities found between the base protons and the H-2′/H-2″ proton region (Fig. 4 middle part). The H-6 signal from T_1 and T_{17} shows cross-peaks to two signals at 2.25 and 1.84 ppm, which must be the corresponding H-2′ and H-2″ proton signals, as there are no 5′ neighbouring residues in the sequence. The adenine protons at 8.476 ppm also have two cross-peaks to these two sugar proton resonances but in addition two stronger ones to resonances at 3.05 and 2.87 ppm. The latter two therefore belong to the same nucleotidyl unit as the resonance at 8.476 ppm, thus establishing the 5′ *T-A* relation.

Next in sequence are T_3 and T_{15}. Their base protons can be identified in two ways, first by the shared NOE to H-1′ of adenines 2 and 16, second by the strong inter-residue NOE between the adenine H-8 protons and the thymidine methyl groups. Both ways led to two H-6 signals at 7.271 and 7.287 ppm, which belong to T_3 and T_{15}. Again this finding is in agreement with the one derived from the H-2′, H-2″ region. At this point of the assignment procedure it was not yet possible to decide which of the two signals belongs to which of the two thymidine residues T_3 and T_{15}.

The next bases towards the 3′ ends are cytidines in both strands. Again their H-6 signals can be assigned via two different cross-relaxation networks, one involving their shared NOE with the two H-1′ protons of thymidine 3 and 15, the other the NOE between the H-6 protons of T_3 and T_{15} and the two H-5 protons at 5.66 and 5.59 ppm. In the next step there is an adenine, A_5, adjacent to C_4 in the upper strand and a cytidine, C_{13}, adjacent to C_{14} in the lower strand. To find a shared cross-peak between C_4 H-1′ and A_5 H-8 or C_{14} H-1′ and C_{13} H-6 we had to rely on information obtained from 2-*D* NOESY experiments with shorter mixing times, because the contour plot of the data collected with 350 ms mixing time contained only the cross-peak to adenine 5′s own H-1′ proton. Another difficulty arose from the strong intensity of the inter-residue cross-peaks between *C* H-6 and *C* H-5 of both cytidines which cover everything else in this spectral region.

From a NOESY experiment with a 100 ms mixing time (spectra not shown) we are able to locate H-1′ of C_4 at 5.57 ppm and H-1′ of C_{14} at 5.92 ppm. At 100 ms mixing time, only protons very close to each other gave rise to a cross-peak, e.g. base protons and H-1′ sugar protons of their 5′ neighbouring residue or H-6 and H-5 protons from the same cytidine residue. This caused a substantial reduction of the number of cross-peaks and helped to locate connectivities in crowded spectral regions. As was the case for the H-1′ region, no cross-peaks could be found between A_5 H-8 and H-2′, H-2″ of C_4 at 350 ms mixing time, but an experiment with 200 ms mixing time gave the necessary information. Although the NOE cross-peaks between C_{14} and C_{13} could not be identified because of severe overlapping of their resonances, two chains of NOE connectivities were identified: $T_1A_2T_3C_4A_5$ and $T_{17}A_{16}T_{15}C_{14}C_{13}$. The knowledge about this conjunction made it possible to distinguish not between the two resonances for T_3 and T_{15}.

To extend the assignment through C_{13} in the lower strand is difficult because of the limited resolution among the H-5 and H-1′ protons of C_4, C_{14}, C_{13}, and C_{12}. It is helpful than to have an independant structural landmark to start anew. As mentioned before, C_{12}-T_{11} is the only 5′ *C-T* step in the O_{R^3} sequence and therefore, could easily be identified by the cross-peak between the C_{12} H-6 and the T_{11} methyl protons (Fig. 4, right part). For the upper strand, no such unique structural landmarks exists. Nevertheless is was possible to assign completely the upper as well as the lower strand by using the sequential assignment rules together with additional information obtained from the COSY spectrum.

Figure 5 shows a portion of the NOESY spectrum containing cross-peaks between the H-1′ protons and the H-2″ and H-2′ protons of the same nucleotidyl unit. These connectivities provided a consistency test

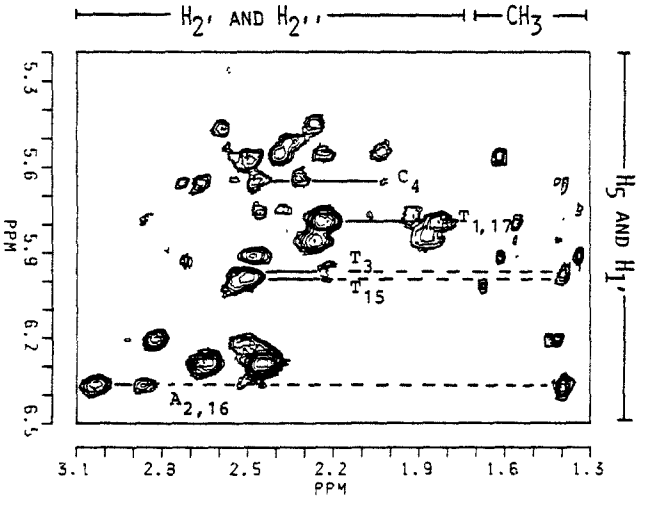

Fig. 5. Intrabase NOE connectivities between the sugar H-1′ and H-2″/H-2′ protons (at 350 ms mixing time). As example the cross-peaks between the H-1′ and the H-2″/H-2′ protons of T_1, T_{17}, A_2, A_{16}, T_3, T_{15}, and C_4 are indicated

for the assignment of the sugar protons which was independently derived from the observation of cross-peaks between the aromatic H-8 or H-6 protons and the particular sugar region. Additional cross-peaks in this region are caused by dipole–dipole interaction between the H-1′ proton of one nucleotidyl unit and the methyl group of a 3′ neighbouring thymidine residue (e.g. between $A_{2,16}$ and CH_3 of $T_{3,15}$). They too were helpful in establishing the position of some H-1′ resonances.

Assignment of H-2 resonances

While the assignment of all the H-8, H-6, H-5, H-1′, H-2″, and H-2′ proton resonances by the sequential assignment procedure is comparatively straightforward, the assignment of the adenosine H-2 protons in D_2O solution is more complicated. In D_2O, H-2 protons have few nearby spins with which to interact e.g. in standard *B*-DNA the nearest H-1′ protons in the minor groove are 0.45–0.5 nm away. Therefore, only weak dipole-dipole interactions are to be expected between H-2 protons and the H-1′ protons of its own sugar, of its 3′ neighbouring sugar, and of its 5′ complementary sugar (Weiss et al. 1984b; Clore and Gronenborn 1984). Normally only the strongest of them, namely the one between the H-2 proton and the H-1′ proton of its 3′ neighbour can be detected. Nevertheless, as the assignment of the H-1′ resonances are obtainable from the sequential assignment procedure, the H-2/H-1′ relationships enable the assignment of the H-2 resonances too.

In the case of *T-A* sequences, additional information is available to group the H-2 resonances. From the geometry of *B*-DNA it follows that for 5′ *T-A* steps but not for 5′ *A-T* steps, the successive H-2 protons will be sufficiently close to permit large NOE's to be observed. The only 5′ *T-A* steps in the O_{R3} sequence occur at the ends, 5′ $T_1A_2T_3$ and 5′ $T_{17}A_{16}T_{15}$. Therefore, only two strong inter – H-2 NOE's should be observed. Figure 6 shows the part of the NOESY spectrum where the cross-peaks among the aromatic resonances are expected to appear. Only two cross-peaks are observed, corresponding to NOE connectivities between A_1 H-2 and A_2 H-2 and between A_{17} H-2 and A_{16} H-2. This information did not allow us to distinguish between them but that was finally done through the weak NOE connectivities to the H-1′ proton of their respective 3′ neighbouring residue. These connectivities also served to assign the remaining five adenosine H-2 resonances which were already identified in H_2O solution by their intra-base pair NOE connectivities to the corresponding imino protons (Chou et al. 1983). No unambiguous assignment could be made for the two H-2 signals at 7.840 and

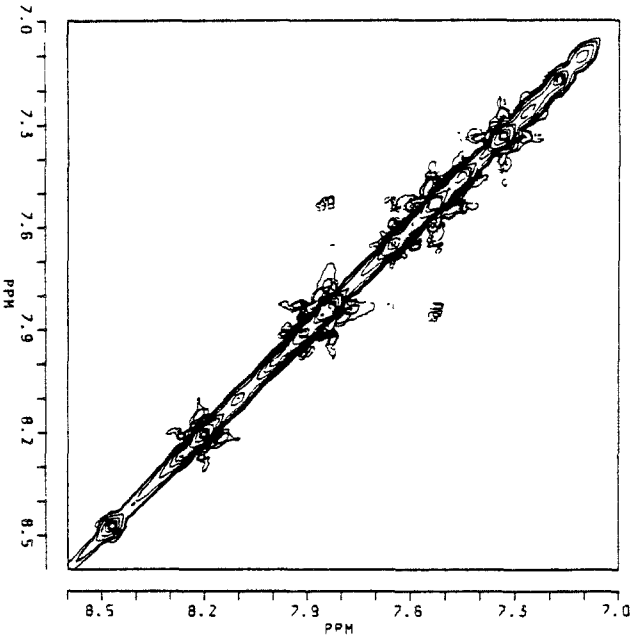

Fig. 6. Portion of the NOESY spectrum with 350 ms mixing time showing the H-2/H-2 cross-peaks between adenines A_1 and A_2 and between adenines A_{17} and A_{16} of the O_{R3} operator DNA

7.921 ppm. Because of their NOE connectivities to the H-1′ protons of T_2 and T_{16} it is obvious that one signal belongs to H-2 of A_{15} and the other to H-2 of A_3 but the overlap of the H-1′ resonances of both thymidines precludes a final assignment.

Summary of the results

In Table 1, the assignment of the H-8, H-6, H-2, and H-5 base protons, the methyl protons and the H-1′, H-2″ and H-2′ sugar protons are given. The sequential assignment procedure yields the position of the H-2′ and H-2″ resonances in a pairwise manner. A discrimination between them might then be done by taking into account the different intensities of their intra-nucleotide NOE interaction with the aromatic of H-1′ proton in right-handed *B*-DNA. While the intra-nucleotide separation between the H-8/H-6 and H-2′ protons is always smaller than that between the H-8/H-6 and H-2″ protons, the reverse holds for the intra-nucleotide separation between the H-1′ and H-2′/H-2″ protons. There, the distance between the H-1′ and H-2″ protons is usually smaller than the distance between the H-1′ and H-2′ protons. A quantitative analysis of cross-peaks intensities or build-up rates of cross-peak intensities should give the necessary information. The absolute value representation together with rather strong resolution enhancement procedures used in our experiments restricted such a quantitative analysis. Even so, a comparison of the distribu-

Table 1. Chemical shifts of assigned ^{1}H resonances in the O_{R3} operator

Residue	H6/H8	H2	H5 or CH_3	H1′	H2″	H2′
Upper strand						
5′ T_1	7.345	–	1.57	5.79	2.25	1.84
A_2	8.476	7.848	–	6.37	3.05	2.87
T_3	7.271	–	1.41	5.98	2.51	2.10
C_4	7.521	–	5.66	5.57	2.38	2.04
A_5	8.274	7.658	–	6.21	2.84	2.71
C_6	7.245	–	5.24	5.80	2.35	1.98
C_7	7.352	–	5.47	5.51	2.32	1.98
G_8	7.838	–	–	5.84	2.75	2.52
C_9	7.268	–	5.36	5.85	2.19	1.86
A_{10}	8.183	7.184	–	5.79	2.85	2.63
A_{11}	8.004	7.457	–	5.90	2.78	2.54
G_{12}	7.468	–	–	5.48	2.57	2.42
G_{13}	7.488	–	–	5.65	2.69	2.47
G_{14}	7.594	–	–	5.66	2.70	2.42
A_{15}	8.106	7.921 (7.840)	–	6.21	2.84	2.56
T_{16}	7.121	–	1.43	5.85	2.28	1.87
A_{17}	8.208	7.556	–	6.27	2.46	2.65
Lower strand						
3′ A_1	8.213	7.540	–	6.29	2.46	2.65
T_2	7.122	–	1.47	5.85	2.27	1.87
A_3	8.164	7.840 (7.921)	–	6.21	2.84	2.55
G_4	7.860	–	–	5.60	2.76	2.69
T_5	7.104	–	1.35	5.75	2.39	1.96
G_6	7.654	–	–	5.93	2.74	2.48
G_7	7.812	–	–	5.63	2.74	2.66
C_8	7.236	–	5.28	5.65	2.33	1.90
G_9	7.860	–	–	5.81	2.75	2.65
T_{10}	7.337	–	1.68	5.78	2.38	2.08
T_{11}	7.454	–	1.63	6.02	2.57	2.19
C_{12}	7.553	–	5.57	5.92	2.48	2.13
C_{13}	7.548	–	5.61	5.45	2.25	1.86
C_{14}	7.586	–	5.59	5.92	2.52	2.17
T_{15}	7.287	–	1.41	5.99	2.51	2.16
A_{16}	8.476	7.872	–	6.37	3.05	2.87
T_{17}	7.345	–	1.57	5.79	2.25	1.84

All chemical shifts are relative to TSP at 25 °C with an accuracy of +/− 0.01 ppm for all aromatic and methyl protons and +/− 0.05 ppm for all H1′, H2″ and H2′ protons

tion of intensities between H-2′ and H-2″ obtained from experiments run with different mixing times made it possible to discriminate between the two types of protons. In the case of ambiguities, the more downfield resonance was attributed to the H-2″ proton, because it is known that the H-2″ protons generally resonate downfield from the H-2′ protons with the exception of those of the 3′ terminal nucleotides (Scheek et al. 1984; Weiss et al. 1984a; Clore and Gronenborn 1984).

Concluding remarks

The assignment of the base and most of the sugar resonances in O_{R3} operator is a first step towards the elucidation of the solution structure of free and repressor-bound DNA. The enormous resolution enhancement attainable in the two-dimensional experiment made such an assignment feasible even for molecules with molecular weights above 10,000 daltons like O_{R3}. The overall regularity of the DNA structure allowed the development of general assignment strategies based on through space NOE connectivities. Apart from the resonance assignment, the agreement between the experimentally observed in the predicted pattern of cross-relaxation connectivities enables one to define the low resolution structure of this particular DNA fragment on a qualitative basis. Nevertheless we refrained from doing so since the absolute value calculation of the spectra and the subsequent resolution enhancement procedure necessary can lead to severe distorsions of the NOE cross-peak intensities. The future use of phase-sensitive two-dimensional spectroscopy in conjunction with a systematic variation of the mixing time in the NOESY experiment to get initial NOE buildup curves seems more promising to us for quantitative evaluations. Experiments are currently being carried out to obtain inter-proton distances from such experiments. This type of study will make it possible to deduce the time averaged spatial configuration of DNA in solution and to detect structural changes in the DNA due to bound ligands.

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Note added in proof:

After submission of the manuscript B. Reid and his coworkers at the University of Washington, Seattle, published a similar NMR study of O_{R3} (Wemmer DE, Chou SH, Reid BR (1984) J Mol Biol 180:41–60). Although some of the experimental conditions were slightly different, essentially the same results were obtained.