O_R3 operator of bacteriophage λ in a 23 base-pair DNA fragment: Sequence-specific ¹H NMR assignments for the non-labile protons and comparison with the isolated 17 base-pair operator

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Abstract. Sequence-specific ¹H NMR assignments are presented for a non-selfcomplementary 23-base-pair DNA duplex of molecular weight 15,000 daltons, containing the O_R3 repressor binding site of bacteriophage λ as the central core. The NMR techniques used were mainly phase-sensitive two-dimensional NOE and 2Q spectroscopy, the latter to overcome overlap problems within the spectral region of the deoxyribose spin-systems. Direct sequential NOE connectivities are observed between adenine 2H and deoxyribose 1' protons. We propose the use of these connectivities as a check of the assignments of C1' and A2 protons, which have independently been derived via other assignment pathways.

Key words: Nuclear magnetic resonance, O_R3 operator, bacteriophage λ

Introduction

Recently we reported a ¹H NMR study in H₂O solution, in which we determined the chemical shifts for the labile protons and most adenine 2H protons in a 23-bp DNA fragment from bacteriophage λ . This duplex contained the 17-bp O_R3 repressor binding site in positions 4–20 (Otting et al. 1987). Since the isolated O_R3 binding site had independently been investigated

in H₂O solution (Kirpichnikov et al. 1984; Ulrich et al. 1983) comparison of the two compounds enabled an assessment of the chain termination effects based on the chemical shift differences of the labile protons. These chemical shift studies represent a qualitative approach to the question of how far the chain termination affects the regular duplex structure of the molecule, which has also been studied by other authors (e.g. Patel et al. 1982).

In the present publication we continue this study with the assignment of the ¹H NMR spectrum of the non-labile protons in the 23-bp DNA. Again, the qualitative assessment of chain termination effects can be based on a comparison with an independent study of the 17-bp O_R3 binding site under similar experimental conditions (Wemmer et al. 1984; Hahn et al. 1985). Special interest is focussed on NOE's with adenine 2H (A2H), especially the use of NOE's between A2H and deoxyribose-1'H to check on independently derived sequence-specific resonance assignments for 1'H and A2H.

Materials and methods

The 23-bp DNA duplex of Fig. 3 was synthesized as two single-stranded polynucleotides by a solid-phase phosphotriester method as described previously (Minganti et al. 1985). The sodium salt of the duplex was dissolved in ²H₂O in a 0.05 M phosphate buffer containing 0.1 M NaCl at pH 7.0. The DNA concentration was 3.6 mM in duplex, the measuring temperature 310 K. All 2-dimensional NMR spectra were recorded at 500 MHz in the phase-sensitive mode on a Bruker WM 500 spectrometer. The set of spectra acquired comprised COSY (Aue et al. 1976), RELAYED-COSY (Eich et al. 1982), NOESY (Jeener et al. 1979; Kumar et al. 1980) and 2-dimensional 2Q spectroscopy (Braunschweiler et al. 1983). NOESY spectra were run with mixing times, τ_m , of 20, 50 and

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Abbreviations: COSY: 2-dimensional correlated spectroscopy; NOESY: 2-dimensional nuclear-Overhauser-enhancement spectroscopy; RELAYED-COSY: 2-dimensional relayed coherence transfer spectroscopy; 2Q: two-quantum; ppm: parts per million; 23-bp DNA: d-(ATCTATCACCGCAAGGGATAAAT) · d-(ATTTATCCCTTGCGGTGATAGAT); 17-bp DNA (O_R3): d-(TATCACCGCAAGGGATA) · d-(TATCCCTTGCGGTGATA); d_i(X; Y): intra-residue distance between protons X and Y; d_s(X; Y): sequential distance between protons X and Y located in sequentially neighbouring nucleotides, where the direction from X to Y is always from 5' to 3'

100 ms. The tuned delay of the RELAYED-COSY was 60 ms, and the excitation period of the 2Q experiment was 33 ms. The time domain data size of the 2Q experiment was $t_{1\max}=30$ ms and $t_{2\max}=121$ ms. The corresponding values of the NOESY spectra were $t_{1\max}=40$ ms and $t_{2\max}=91$ ms. Prior to Fourier transformation, the data were multiplied with shifted sine-bell windows. In the 2Q spectrum an unshifted sine-bell was applied along the ω_2 axis.

Results

For the identification of the proton spin systems of the pyrimidine bases and the deoxyribose moieties COSY and 2Q spectroscopy were utilized. The advantage of 2Q spectroscopy over COSY to overcome spectral overlap problems in the assignment of spin-systems in larger proteins was demonstrated by Rance and Wright (1986) and has been applied to the assignment of the 4'H in medium-sized oligonucleotides (Chazin et al. 1986; Hanstock and Lown 1984). Figure 1 shows the remote peak region of the 1' protons in the 2Q spectrum of the 23-bp DNA. With the excitation period used, these peaks are easily distinguished from direct peaks, since they are of opposite sign (Rance and Wright 1986). 44 out of 46 possible remote cross peaks at ($\omega_1 = \omega(2'H) + \omega(2''H)$; $\omega_2 = \omega(1'H)$) are well resolved. By comparison, in the spectral region of the COSY spectrum containing the cross peaks 1'H-2'H and 1'H-2''H only about 60% of the cross peaks could be resolved (data not shown), which demonstrates the superiority of 2Q spectroscopy over COSY for the identification of the 1', 2' and 2'' proton resonances in the 23-bp DNA. Since the chemical shifts of the 1' and 2' protons are known either from the 1'H-2'H COSY cross peak or the direct connectivity at ($\omega_1 = \omega(1'H) + \omega(2'H)$, $\omega_2 = \omega(1'H)$) in the 2Q spectrum, the chemical shift of the 2'' proton can be easily calculated from the ω_1 -frequency of the corresponding remote peak (Fig. 1).

The 3' protons could partly be assigned using the RELAYED-COSY technique (Chazin et al. 1986). Our experiment, however, yielded only about one third of the expected 1'H-3'H relay cross peaks. For the assignment of all other 3'H, we employed the $d_i(6, 8; 3')$ and $d_s(3'; 6, 8)$ cross peaks of a NOESY experiment recorded with a mixing time 100 ms, where spin-diffusion (Kalk and Berendsen 1976; Wagner and Wüthrich 1979) is active (Chazin et al. 1986; Wüthrich 1986). At this mixing time any base proton 6 or 8 exhibits NOE's to two 3' protons. The stronger cross peak is due to the connectivity $d_i(6, 8; 3')$, the weaker one to $d_s(3'; 6, 8)$, whereby the latter cross peak vanishes in NOESY spectra recorded with mixing times shorter than 50 ms. We were thus able to deter-

Fig. 1. Region of the 500 MHz phase-sensitive 2Q spectrum of the 23-bp DNA of Fig. 3 recorded with an excitation period of 33 ms, showing the remote peaks connecting the 1'H (ω_2) with the 2'H and 2''H (ω_1). Cross peaks appear at ($\omega_1 = \omega(2'H) + \omega(2''H)$, $\omega_2 = \omega(1'H)$). All cross peaks are labeled with the one-letter-code of the nucleotide and its sequence number. Asterisks indicate unidentified cross peaks due to impurities

mine the chemical shifts of all 3'H's, which strongly overlap. Note, however, that the assignment of the 3'H's depended on the sequential resonance assignments of the 6H and 8H of the bases, which had to be accomplished first. Since the resonance positions of the 4' protons overlap strongly with those of 5' and 5'' protons, and since some of the sequential distances $d_s(1'; 5', 5'')$ might be shorter than the intra-nucleotide distance $d_i(1'; 4')$ (Wüthrich 1986; Celda et al. 1988), we abandoned the assignment of the 4' protons by NOESY.

For the sequential resonance assignments of the base protons we used primarily the spectral region of a NOESY spectrum ($\tau_m=100$ ms) containing the NOESY cross peaks between base protons 6 or 8 and sugar protons 1' (Hare et al. 1983; Scheek et al. 1983; Scheek et al. 1984). This was advantageous, since the base protons and the 1' protons have the largest chemical shift dispersion of all non-labile protons in DNA, and therefore, the observation of the NOE's corre-

Fig. 2A and B. Region of the 500 MHz absorption mode NOESY spectrum of the 23-bp DNA of Fig. 3 recorded with 100 ms mixing time, showing the sequential connectivities $d_i(6, 8; 1')$ followed by $d_s(1'; 6, 8)$ for the (+)-strand (A) and the (-)-strand (B), respectively. Intra-residue cross peaks are labeled with the nucleotide number. Cross peaks between A2H and 1'H are labeled with frames and connected by broken lines if they involve the same A2H. Cross peaks due to $d_s(6, 8; 5)$ are connected to either the cross peaks $d_i(6, 8; 1')$ or $d_s(1'; 6, 8)$ of the same 6 or 8 proton by dashed-dotted vertical lines. The resonance positions of the A2H's are indicated at the bottom of the figure. The strong cross peaks $d_i(5; 6)$ of the cytosines are identified at the right side for the 5H and at the bottom for the 6H. The start of the assignment pathway at the 5' end is indicated by an arrow

sponding to $d_i(6, 8; 1')$ and $d_s(1'; 6, 8)$ is easier than for the cross peaks involving 2'H and 2''H.

The two 5'-terminal adenine 8 protons (A8H) are degenerate and identified as the terminal ones by two criteria: First, there are only two NOESY cross peaks to 2'H and 2''H and only one cross peak to 1'H, corresponding to the direct NOE connectivities $d_i(8; 2')$, $d_i(8; 2'')$ and $d_i(8; 1')$, respectively. Second, we observed a considerably faster $^1\text{H}-^2\text{H}$ exchange rate of the 5'-terminal A8H's than for the other A8H's (Benevides et al. 1984; Brandes and Ehrenberg 1986). The 3'-terminal thymidine 6 protons (T6H) exhibit a significantly smaller linewidth than the other T6H's, the remote peaks in the 2Q spectrum are stronger, the 2'H and 2''H resonances are degenerate, and the resonance

position of the 3' protons is at higher field because the compound contains no terminal phosphate groups.

The sequential resonance assignment of the (+)-strand (Fig. 2A) starts with the single cross peak involving the 8H of the 5'-terminal A_1 (arrow in Fig. 2A). To this intra-residue connectivity $d_i(8; 1')$ we connect T_2 via the NOESY cross peak $d_s(1'; 6)$. Following the lines in Fig. 2A the assignment is straightforward up to residue T_6 , using always the connectivities $d_i(6, 8; 1')$ followed by $d_s(1'; 6, 8)$. Whereas there is no connection between T_6 and C_7 via $d_s(1'; 6)$, these nucleotides can be connected via $d_s(6; 5)$. $d-C_7A_8C_9$ is a unique trinucleotide in the 23-bp DNA, whereby the resonance position of A_8H is immediately identified through the NOESY cross peak $d_s(8; 5)$ between A_8

Table 1. Chemical Shifts (± 0.02 ppm) of all assigned non-labile protons of the 23-bp DNA of Fig. 3. Shifts were measured in $^2\text{H}_2\text{O}$ solution, $T = 310$ K, 0.1 M NaCl, 0.05 M phosphate buffer, pH 7.0. They are indirectly referenced to 2,2-dimethyl-2-silapentane-5-sulphonate using the HO^2H resonance previously calibrated in stock buffer solution

Residue	8	6	5	5-CH ₃	2	1'	2'	2''	3' ^a
<i>(A) (+)-strand</i>									
A ₁	8.17				7.93	6.23	2.69	2.84	4.87
T ₂		7.39		1.37		6.04	2.23	2.52	4.85
C ₃		7.60	5.64			6.01	2.13	2.50	(4.76)
T ₄		7.41		1.67		5.69	2.17	2.50	(4.90)
A ₅	8.31				7.30	6.25	2.65	2.90	(5.02)
T ₆		7.11		1.36		5.84	1.97	2.38	(4.82)
C ₇		7.44	5.61			5.51	1.97	2.33	(4.81)
A ₈	8.21				7.60	6.15	2.66	2.83	(5.00)
C ₉		7.21	5.23			5.77	1.92	2.33	4.86
C ₁₀		7.32	5.46			5.52	1.92	2.30	(4.78)
G ₁₁	7.81					5.82	2.54	2.66	(4.94)
C ₁₂		7.23	5.36			5.46	1.80	2.21	4.76
A ₁₃	8.15				7.16	5.77	2.66	2.80	(5.00)
A ₁₄	7.97				7.42	5.87	2.51	2.72	(5.00)
G ₁₅	7.44					5.47	2.36	2.55	(4.90)
G ₁₆	7.46					5.63	2.39	2.65	(4.90)
G ₁₇	7.55					5.60	2.46	2.67	(4.94)
A ₁₈	8.05				7.70	6.15	2.49	2.83	4.96
T ₁₉		7.03		1.32		5.53	1.82	2.22	4.82
A ₂₀	8.12				6.78	5.80	2.60	2.78	(5.01)
A ₂₁	8.00				7.14	5.86	2.56	2.78	(5.02)
A ₂₂	8.01				7.74	6.14	2.60	2.76	4.94
T ₂₃		7.10		1.37		6.06	2.11	2.11	4.49
<i>(B) (-)-strand</i>									
A ₋₂₃	8.17				7.89	6.24	2.70	2.85	4.87
T ₋₂₂		7.39		1.37		6.08	2.18	2.55	(4.88)
T ₋₂₁		7.48		1.61		6.14	2.17	2.58	(4.89)
T ₋₂₀		7.41		1.69		6.24	2.16	2.52	(4.89)
A ₋₁₉	8.31				7.29	6.24	2.65	2.90	(5.02)
T ₋₁₈		7.13		1.33		5.86	2.04	2.42	(4.82)
C ₋₁₇		7.50	5.55			5.89	2.08	2.46	4.75
C ₋₁₆		7.48	5.52			5.92	2.15	2.43	4.78
C ₋₁₅		7.50	5.55			5.88	2.16	2.43	4.79
T ₋₁₄		7.40		1.60		5.99	2.07	2.50	(4.88)
T ₋₁₃		7.29		1.64		5.75	2.05	2.40	4.85
G ₋₁₂	7.83					5.79	2.54	2.66	(4.95)
C ₋₁₁		7.21	5.26			5.64	1.80	2.28	(4.77)
G ₋₁₀	7.78					5.62	2.61	2.71	(4.94)
G ₋₉	7.58					5.89	2.41	2.67	(4.88)
T ₋₈		7.05		1.30		5.73	1.89	2.34	(4.82)
G ₋₇	7.82					5.58	2.63	2.73	(4.98)
A ₋₆	8.15				7.62	6.17	2.55	2.84	(4.98)
T ₋₅		7.07		1.35		5.54	1.88	2.26	4.82
A ₋₄	8.08				7.18	5.95	2.61	2.79	(5.01)
G ₋₃	7.59					5.50	2.47	2.61	(4.95)
A ₋₂	8.07				7.89	6.25	2.55	2.80	(4.93)
T ₋₁		7.22		1.42		6.12	2.14	2.14	4.50

^a The ^3H resonances in parentheses were assigned on the basis of NOESY connectivities $d_i(6,8;3')$ and $d_s(3';6,8)$ (for details cf. Results)

and C₉. This connects C₇ to A₈ via $d_s(1';8)$ and from there on the connectivity scheme $d_i(6,8;1')$ followed by $d_s(1';6,8)$ can be applied up to the $d_i(8;1')$ NOESY cross peak of residue G₁₅. The shape of this cross peak indicates that the chemical shifts of the 8H of G₁₅ and

G₁₆ are nearly identical. The gap between G₁₅ and G₁₆ cannot be closed by any other sequential connectivity. The sequential resonance assignment can be continued by starting with the NOESY cross peak $d_i(8;1')$ of T₂₃ and pursuing the assignment in the

direction from the 3' end to the 5' end. One readily arrives at the NOESY cross peak $d_i(8; 1')$ of G_{16} , thus completing the assignment of the (+)-strand.

The sequential resonance assignments for the (−)-strand (Fig. 2B) starts at the NOESY cross peak $d_i(8; 1')$ of A_{-23} (arrow in Fig. 2B). From there one easily arrives at the NOESY cross peak $d_i(6; 1')$ of T_{-18} . Although there is no resolved cross peak $d_s(1'; 6)$ between T_{-18} and C_{-17} , an unambiguous NOESY cross peak $d_s(6; 5)$ connects these two residues. The sequence $d-C_{-15}T_{-14}T_{-13}$ is unique in the duplex and can be linked together via $d_s(1'; 6)$. C_{-16} is attached to G_{-15} via a NOESY cross peak $d_s(2''; 6)$, observed in a NOESY recorded with a mixing time $\tau_m = 20$ ms (data not shown). T_{-13} up to G_{-10} are easily connected via $d_i(6, 8; 1')$ and $d_s(1'; 6, 8)$. In addition, the connectivity $d_s(1'; 8)$ between G_{-12} and C_{-11} is confirmed by $d_s(8; 5)$ between these nucleotides. Since G_{-9} could not be linked to G_{-10} by a NOE connectivity, a second assignment pathway was started at the cross peak $d_i(6; 1')$ of T_{-1} in the direction from 3' toward the 5' end. This pathway leads directly to the cross peak $d_i(8; 1')$ of G_{-9} , thereby completing the sequence-specific resonance assignments of the (−)-strand. Despite the fact, that the first seven residues of each DNA strand were identical in all but one residue, we did not have difficulties in following the assignment pathway along one strand.

Additional studies concentrated on the adenine 2 protons. Initially these A2H resonances were distinguished from the 8H resonance lines of A and G by the fact, that in a sample which was heated for 60 h at 328 K, the A8H and G8H got exchanged by deuterium to a high degree (Benevides et al. 1984; Brandes and Ehrenberg 1986), whereas the A2H remained. Direct NOESY cross peaks due to the sequential intra-strand connectivity $d_s(2; 2)$ between A2H's have been observed for the dinucleotide segments $A_{13}A_{14}$, $A_{20}A_{21}$, and $A_{21}A_{22}$. Inter-strand A2H-A2H NOE's between neighboring base pairs were observed for the duplex segment 18–19. The remaining cross peaks of this type (see Fig. 3) could not be identified. The A2H resonances of A_{-4} , A_5 , A_{-6} and A_8 were previously assigned via the intra-base-pair and sequential NOE's with imino protons (Otting et al. 1987). The sequence-specific resonance assignments of the remaining 2-protons of A_{-1} , A_{-2} and A_{23} were obtained using the NOE's between A2H and 1' protons (see below).

For all A2H's in the 5'-adenine-pyrimidine-3'-dinucleotides 5'-dAdT-3' and 5'-dAdC-3' we observe a NOESY cross peak (recorded at $\tau_m = 100$ ms) corresponding to the sequential intra-strand connectivity $d_s(2; 1')$. The same connectivity also gives rise to a cross peak between A_{21} 2H and A_{22} 1'H. The NOESY cross peaks due to inter-strand distances between A2H and the 1'H of the sugar on the 3' side of its comple-

Fig. 3. Survey of the sequential NOE's observed in NOESY spectra of the 23-bp DNA. Heavy lines indicate direct NOE's which can be observed without spin-diffusion contributions. The light lines indicate connectivities observable only at mixing times where spin-diffusion is effective. The connectivities are given at the top for the (+)-strand and at the bottom for the (−)-strand. Inter-strand connectivities between A2H and 1'H are not indicated here (cf. Results). A sequential connectivity is only indicated, if the corresponding NOESY cross peak could be assigned unambiguously

mentary nucleotide are observed for all six 5'-purine-adenine-3' dinucleotide units in the duplex. For A2H of residue 13, we were not able to distinguish between intra-residue and inter-strand NOE, since the chemical shifts of the 1'H of G_{-12} and A_{13} are nearly degenerate. A strong intra-nucleotide NOESY cross peak $d_i(2; 1')$ is observed for the two terminal adenines, whereas at a mixing time of $\tau_m = 100$ ms these cross peaks are very weak for A_{18} , A_{-19} , A_{20} , A_5 and A_{-6} . The shape of the NOESY cross peak $d_i(6; 1')$ of T_{-18} suggests the presence of an overlapping NOESY cross peak due to $d_i(2; 1')$ for A_{21} or to $d_s(2; 1')$ between A_{13} and A_{14} , but the near-degeneracy of the 1'H chemical shifts of A_{14} and A_{21} prevented any conclusive analysis. The positions of possible cross peaks $d_i(2; 1')$ for residues A_{14} and A_{22} were masked by much stronger cross peaks and thus could not be analyzed. The cross peaks linking A2H of A_{20} with 1'-deoxyribose protons were too weak to provide reliable data. A_{-2} , A_{-4} and A_8 did not exhibit an intra-nucleotide NOE $d_i(2; 1')$, and for A_{-4} the NOE corresponding to $d_s(2; 1')$ was also missing.

Discussion

While we performed the sequential resonance assignments in the spectral region with the least spectral overlap, using the connectivities $d_i(6, 8; 1')$ and $d_s(1'; 6, 8)$ in a NOESY spectrum recorded with $\tau_m = 100$ ms, we also experimented with the use of other sequential resonance assignment schemes (see Chazin et al. 1986) and refs. therein) for this relatively large oligonucleotide. Assignments with combined use of COSY and NOESY involving 2'H and 2''H were

limited because of cancellation of some of the anti-phase COSY cross peaks. However, the missing information could be collected from a 2-dimensional 2Q experiment (Fig. 1). With this additional information it became possible to obtain numerous sequential resonance assignments with a COSY-NOESY connectivity diagram as proposed by Chazin and coworkers (Chazin et al. 1986), using a NOESY spectrum recorded with $\tau_m = 20$ ms (data not shown). In this NOESY spectrum only direct sequential NOE's between closely spaced protons were observed, namely $d_s(2'; 6, 8)$ and $d_s(5, 6, 8; 5\text{-CH}_3)$. A summary of all observed sequential NOE's in the 23-bp DNA is given in Fig. 3.

There exist four continuous, independent pathways for homonuclear sequential resonance assignments of protons in double-stranded DNA (Wüthrich 1986). One pathway can be followed in H_2O solution only, whereas the other three are best followed in $^2\text{H}_2\text{O}$ solution. There exist connections between the assignment pathways in H_2O and $^2\text{H}_2\text{O}$, which can be used to cross-check the independently derived resonance assignments. In a NOESY spectrum taken in H_2O solution under conditions of extensive spin-diffusion, there occur cross peaks between imino protons and cytosine 5H and thymidine 5- CH_3 resonances (e.g., (Boelens et al. 1985)). In the case of adjacent A · T base pairs one can check the assignment of the adenine 2 protons derived from NOE's between imino protons and A2H in a NOESY spectrum in H_2O by the connectivity $d_s(2; 2)$ observed in $^2\text{H}_2\text{O}$. Here we also used $d_s(2; 1')$ and inter-strand A2H-1'H NOE's as a check of the proton resonance assignments made via $d_i(6, 8; 1')$ and $d_s(1'; 6, 8)$, and as an alternative way to sequentially assign the A2H's. We were thus able to confirm all the previous assignments of the A2H. The assignments of the A2H's of A_{-1} , A_{23} and A_{-2} were done solely on the basis of these connectivities.

The occurrence of NOESY cross peaks due to intra- and inter-strand A2H-1'H connectivities is unexpected when considering the ^1H - ^1H distances derived from X-ray diffraction data of DNA fibers (Arnott, personal communication and (Wüthrich 1986)), which are all larger than 0.5 nm. However, in single crystals of oligonucleotides one finds distances of A2H to adjacent 1'H's as short as 0.4 nm. From published single crystal X-ray structures (Dickerson and Drew 1981; Conner et al. 1984; Drew et al. 1981; Drew et al. 1982; Fratini et al. 1982; Wing et al. 1984; Shakked et al. 1983) we computed the range of distances A2H-1'H. $d_i(2; 1')$ was thus found to be between 0.43 and 0.47 nm with an average of 0.45 nm. $d_s(2; 1')$ in 5'-dAdT-3' and 5'-dAdC-3' dinucleotides varies between 0.36 and 0.43 nm averaging around 0.39 nm. In the 5'-dAdA-3' dinucleotide this distance is between 0.41 and 0.46 nm, on average about 0.43 nm

(in the available X-ray structures there was no 5'-dAdG-3' sequence). The inter-strand distance $d_s(2; 1')$ between A2H and the 1'H on the 3' side of its complementary nucleotide was found to be 0.4 nm for all sequences studied, with a variation between 0.33 and 0.46 nm. NOE's corresponding to $d_i(2; 1')$, $d_s(2; 1')$ and the inter-strand A2H-1'H distance were previously observed by Weiss et al. (1984) in a NOESY spectrum recorded with $\tau_m = 300$ ms of the O_L1 repressor binding site of bacteriophage λ , i.e. d-(TATCCACTGGCGGTGATA) · d-(TATCACCGC-CAGTGGATA). They also used these cross peaks to assign some of the A2H resonances.

In the NOESY spectra of the 23-bp DNA recorded with $\tau_m = 100$ ms we observed a systematic variation of the intensities of the NOESY cross peaks between A2H and 1'H. For all 5'-dAdC-3' and 5'-dAdT-3' dinucleotide repeats we found a strong NOE corresponding to $d_s(2; 1')$, and for all 5'-dGdA-3' and 5'-dAdA-3' dinucleotides a strong sequential, inter-strand NOE was observed. The intra-nucleotide NOE $d_i(2; 1')$ was generally weaker or not observed, except in the terminal adenines, where its occurrence might be a consequence of the higher mobility of the terminal nucleotides. Inspection of molecular models makes clear that the sequential distance $d_s(2; 1')$ must be very sensitive to local structural variations arising from base roll, propeller twist or sliding movements along the long base pair axes, which also affect the width of the minor groove (Fratini et al. 1982; Dickerson 1983; Alexeev et al. 1987). We can therefore conclude from the present observation that there are sequence-dependent variations of the local conformation along the duplex formed by the 23-bp DNA. Similar variations of the A2H-1'H NOE intensities were also observed in other DNA duplexes (Celda et al. 1988; Kintanar et al. 1987). For the future the use of these NOE's might develop further as probes of the local DNA structure. Because A2H is isolated from other non-labile protons, the contribution of spin-diffusion is minimal for A2H-1'H NOE's, so that they should be useful for studies of structural features in duplexes even when measured in NOESY spectra recorded with long mixing times.

The 23-bp DNA under study contains the 17-bp DNA of the O_R3 repressor binding site of bacteriophage λ as the central core. The non-labile protons of the isolated 17-bp DNA were also assigned (Wemmer et al. 1984; Hahn et al. 1985). Figure 4 shows the differences in chemical shift between the 17-bp O_R3 repressor binding site and the 23-bp DNA for the base 6, 8 and the deoxyribose 1' protons, respectively. These changes in chemical shifts reflect chain end effects, since they are sensitive to both the chemical nature of the additional base-pairs and to the conformational changes introduced by the added nu-

Fig. 4. Plot of the chemical shift differences of corresponding protons versus the sequence in the 23-bp DNA and the 17-bp DNA containing the O_R3 repressor binding site of bacteriophage λ . The upper box shows the difference $\Delta\delta_{6,8}$ between corresponding base protons 6H and 8H, and at the bottom $\Delta\delta_{1'}$ between corresponding sugar protons 1'H is given (•: (+)-strand, x: (-)-strand). For the (+)-strand the numeration is indicated at the top, for the (-)-strand at the bottom (both strands are written from left to right as going from the 5' to the 3' end). Between the two boxes a schematic drawing shows the location of the 23-bp DNA relative to the 17-bp fragment O_R3. Data for this analysis are from the present measurements for the 23-bp DNA (for measuring conditions cf. Materials and methods) and from Wemmer et al. (1984) for the 17-bp DNA (Spectra taken in ²H₂O solution at 310 K, containing 0.01 M phosphate buffer and 0.1 M NaCl at pH 7.0; the concentration of the DNA was not indicated)

cleotides. The contribution of these two factors to the observed changes in chemical shifts cannot be apportioned. Taking into account that the experiments were done under somewhat different solvent conditions, we consider variations of less than 0.05 ppm to be within the accuracy of the measurements. However, the differences in the ¹H chemical shifts indicate that chain termination effects involve at least the peripheral three base-pairs from either end of the duplex, as also found by other authors (e.g. Patel et al. 1982) for short oligonucleotide duplexes. Chain termination might thus also be one of the reasons for the fact that the structure of d-(ATATATATAT)₂ (Suzuki et al. 1986) or of d-CTGGATCCAG)₂ (Nüges et al. 1987) calculated from NMR data by molecular mechanics or molecular dynamics methods showed locally different structural features at the ends of the duplexes, when compared to the central part.

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References

- Alexeev DG, Lipanov AA, Skuratovski IY (1987) The structure of poly(dA) · poly(dT) as revealed by an X-ray fibre diffraction. *J Biomol Struct Dyn* 4:989–1012
- Aue WP, Bartholdi E, Ernst RR (1976) Two-dimensional spectroscopy. Application to nuclear magnetic resonance. *J Chem Phys* 64:2229–2246
- Benevides JM, Lemeur D, Thomas Jr GJ (1984) Molecular conformations and 8-CH exchange rate of purine ribo- and deoxyribonucleotides: Investigation by Raman spectroscopy. *Biopolymers* 23:1011–1024
- Boelens R, Scheek RM, Dijkstra K, Kaptein R (1985) Sequential assignment of imino- and amino-proton resonances in ¹H NMR spectra of oligonucleotides by two-dimensional NMR spectroscopy. Application to a lac operator fragment. *J Magn Reson* 62:378–386
- Brandes R, Ehrenberg A (1986) Kinetics of the proton-deuteron exchange at position H8 of adenine and guanine in DNA. *Nucleic Acids Res* 14:9491–9508
- Braunschweiler L, Bodenhausen G, Ernst RR (1983) Analysis of networks of coupled spins by multiple quantum NMR. *Mol Phys* 48:535–560
- Celda B, Widmer H, Leupin W, Chazin WJ, Denny WA, Wüthrich K (1988) Studies of the conformation of d-(AAAAATTTT)₂ using constraints from nuclear Overhauser effects and from quantitative analysis of the cross peak fine structures in two-dimensional ¹H nuclear magnetic resonance spectra. *Biochemistry* (in press)
- Chazin WJ, Wüthrich K, Rance M, Hyberts S, Denny WA, Leupin W (1986) ¹H nuclear magnetic resonance assignments for d-(GCATTAATGC)₂ using experimental refinements of established procedures. *J Mol Biol* 190:439–453
- Conner BN, Yoon C, Dickerson JL, Dickerson RE (1984) Helix geometry and hydration in an A-DNA tetramer: ¹C-C-G-G. *J Mol Biol* 174:663–695
- Dickerson RE (1983) Base sequence and helix structure variation in B and A DNA. *J Mol Biol* 166:419–441
- Dickerson RE, Drew HR (1981) Structure of a B-DNA dodecamer: II. Influence of base sequence on helix structure. *J Mol Biol* 149:761–786
- Drew HR, Loing RM, Takano T, Broka C, Tanaka S, Itakura K, Dickerson RE (1981) Structure of a B-DNA dodecamer: Conformation and dynamics. *Proc Natl Acad Sci USA* 78:2179–2183
- Drew HR, Samson S, Dickerson RE (1982) Structure of a B-DNA dodecamer at 16 K. *Proc Natl Acad Sci USA* 79:4040–4044
- Eich G, Bodenhausen G, Ernst RR (1982) Exploring nuclear spin systems by relayed magnetization transfer. *J Am Chem Soc* 104:3731–3732
- Fratini AV, Kopka ML, Drew HR, Dickerson RE (1982) Reversible bending and helix geometry in a B-DNA dodecamer: CGCGAATT^{Br}CGCG. *J Biol Chem* 257:14686–14707
- Hahn KD, Buck F, Rüterjans H, Chernov BK, Skryabin KG, Kirpichnikov MP (1985) ¹H 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. *Eur Biophys J* 12:87–95
- Hanstock CC, Lown JW (1984) Two-dimensional double-quantum spectroscopy for the hydrogen NMR assignment of deoxyribonucleotides. *J Magn Reson* 58:167–172
- Hare D, Wemmer DE, Chou SH, Drobny G, Reid BR (1983) Assignment of the non-exchangeable proton resonances of d-(C-G-C-G-A-A-T-T-C-G-C-G) using two-dimensional nuclear magnetic resonance methods. *J Mol Biol* 171:319–336

- Jeener J, Meier BH, Bachmann P, Ernst RR (1979) Investigation of exchange processes by two-dimensional NMR spectroscopy. *J Chem Phys* 71:4546–4553
- Kalk A, Berendsen HJ (1976) Proton magnetic relaxation and spin diffusion in proteins. *J Magn Reson* 24:343–366
- Kintanar A, Klevit RE, Reid BR (1987) Two-dimensional NMR investigation of a bent DNA fragment: Assignment of the proton resonances and preliminary structure analysis. *Nucleic Acids Res* 15:5845–5862
- Kirpichnikov MP, Hahn KD, Buck F, Rüterjans H, Chernov BK, Kurochkin A, Skryabin KG, Bayev AA (1984) ^1H NMR study of the interaction of bacteriophage λ Cro protein with the O_R3 operator. Evidence for a change of the conformation of the O_R3 operator on binding. *Nucleic Acids Res* 12:3551–3561
- Kumar A, Ernst RR, Wüthrich K (1980) A two-dimensional nuclear Overhauser enhancement (2D NOE) experiment for the elucidation of complete proton-proton cross-relaxation networks in biological macromolecules. *Biochem Biophys Res Commun* 95:1–6
- Minganti C, Ganesh KN, Sproat BS, Gait MJ (1985) Comparison of controlled pore glass and Kieselguhr-polydimethylacrylamide composite as supports for solid-phase synthesis of 23-residue oligodeoxynucleotides in milligram amounts. *Anal Biochem* 147:63–74
- Nilges M, Clore GM, Gronenborn AM, Piel N, McLaughlin LW (1987) Refinement of the solution structure of the DNA decamer 5'd(CTGGATCCAG) $_2$: Combined use of nuclear magnetic resonance and restrained molecular dynamics. *Biochemistry* 26:3734–3744
- Otting G, Grütter R, Leupin W, Minganti C, Ganesh KN, Sproat BS, Gait MJ, Wüthrich K (1987) Sequential NMR assignments of labile protons in DNA using two-dimensional nuclear-Overhauser-enhancement spectroscopy with three jump-and-return pulse sequences. *Eur J Biochem* 166:215–220
- Patel DJ, Kozlowski SA, Marky LA, Broka C, Rice JA, Itakura K, Breslauer KJ (1982) Premelting and melting transitions in the d-(CGCGAATTCGCG) self-complementary duplex in solution. *Biochemistry* 21:428–436
- Rance M, Wright PE (1986) Analysis of ^1H NMR spectra of proteins using multiple quantum coherence. *J Magn Reson* 66:372–378
- Scheek RM, Russo N, Boelens R, Kaptein R, van Boom JH (1983) Sequential resonance assignments in DNA ^1H NMR spectra by two-dimensional NOE spectroscopy. *J Am Chem Soc* 105:2914–2916
- Scheek RM, Boelens R, Russo N, van Boom JH, Kaptein R (1984) Sequential resonance assignments in ^1H NMR spectra of oligonucleotides by two-dimensional NMR spectroscopy. *Biochemistry* 23:1371–1376
- Shakked Z, Rabinovich D, Kennard O, Cruse WB, Salisbury SA, Viswamitra MA (1983) Sequence-dependent conformation of an A-DNA double helix. The crystal structure of the octamer d(G-G-T-A-T-A-C-C). *J Mol Biol* 166:183–201
- Suzuki E, Pattabiraman N, Zon G, James TL (1986) Solution structure of [d(A-T) $_3$] $_2$ via complete relaxation matrix analysis of two-dimensional nuclear Overhauser effect spectra and molecular mechanics calculations: Evidence for a hydration tunnel. *Biochemistry* 25:6854–6865
- Ulrich EL, John EM, Gough GR, Brunden MJ, Gilham PT, Westler WM, Markley JL (1983) Imino proton assignment in the proton nuclear magnetic resonance spectrum of the λ phage O_R3 deoxyribonucleic acid fragment. *Biochemistry* 22:4362–4365
- Wagner G, Wüthrich K (1979) Truncated driven nuclear Overhauser effect (TOE). A new technique for studies of selective ^1H - ^1H Overhauser effects in the presence of spin diffusion. *J Magn Reson* 33:675–680
- Weiss MA, Patel DJ, Sauer RT, Karplus M (1984) ^1H -NMR study of the λ operator site O_L1 : Assignment of the imino and adenine H2 resonances. *Nucleic Acids Res* 12:4035–4047
- Wemmer DE, Chou SH, Reid BR (1984) Sequence-specific recognition of DNA: Nuclear magnetic resonance assignments and structural comparison of wild-type and mutant λ O_R3 operator DNA. *J Mol Biol* 180:41–60
- Wing RM, Pjura P, Drew HR, Dickerson RE (1984) The primary mode of binding of cisplatin to a B-DNA dodecamer: C-C-C-G-A-A-T-T-C-G-C-G. *EMBO J* 3:1201–1206
- Wüthrich K (1986) NMR of proteins and nucleic acids. Wiley, New York, pp 203–255