credence to Winstein's original suggestion[15] that the saturated parent system, **17**, underwent ionization with some anchimeric assistance.

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NMR Characterization of DNA: Assignment of Major Groove Sugar Protons of the λ-Phage Operator Site O_L1 by Two-Dimensional NOE and *J*-Correlated Spectra

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The 17 base-pair DNA operator site O_L1 is recognized by the *c*I and Cro repressors of bacteriophage λ and thus provides a model system for specific protein–DNA recognition.[1] The solution structure and dynamics of this oligonucleotide are being studied by two-dimensional correlated (COSY) and Overhauser (NOESY) proton NMR methods.[2-4] We and others have recently proposed a sequential assignment strategy for oligomers of B DNA.[5-8] This strategy is extended here to include the major groove sugar protons H3′ and H4′ and is illustrated by the assignment of these protons in the oligonucleotide O_L1. The H3′ and H4′ protons are of particular interest because they lie on the outer surface of the double helix and may be involved in ligand binding.

The essential element in this and other DNA assignment strategies[5-8] is that the DNA double helix, unlike some RNAs (e.g., tRNA) and proteins, is a two-dimensional polymer with a characteristic structure. Protons distant in the primary sequence are also spatially separated, and it is possible to predict which proton NOE's are expected to be nonnegligible.[9] Each strand of the B DNA double helix in D_2O solution is found to constitute an independent cross-relaxation network of base and sugar protons. Each deoxyribose sugar contributes to this network a distinct set of spins exhibiting strong mutual cross-relaxation, but only weak interaction with the protons of other sugars. Because DNA is a two-dimensional polymer containing a string of largely independent spin reservoirs, spin diffusion can be an aid rather than a hindrance to sequential assignment.[10]

The H1′ and H2′–H2″ sugar protons and the base protons in the major groove of the O_L1 DNA double helix have been assigned previously by a sequential method.[7,11,12] The assignments of these protons can be used to assign the H3′ and H4′ protons. The H3′ protons may be identified by indirect nuclear Overhauser effects (NOE's) from the base protons and also from the H1′ and H2′–H2″ sugar protons. The assignment of these sugar protons to the same sugar is confirmed by a chain of *J* connectivities from H1′ to H2′ and then from H2′ to H3′ observed in the COSY experiment. The H4′ protons may be identified by NOE's from the H1′ protons; weak indirect effects are sometimes also observed from the base protons. Where resolved, the *J* connectivity between the H3′ and H4′ protons verifies the consistency of these two sets of assignments.[13]

O_L1 is an asymmetric duplex of 17 base pairs with sequence

$$5' \; T_1A_2C_3C_4A_5C_6T_7G_8G_9C_{10}G_{11}G_{12}T_{13}G_{14}A_{15}T_{16}A_{17} \; 3'$$

$$3' \; A_1T_2G_3G_4T_5G_6A_7C_8C_9G_{10}C_{11}C_{12}A_{13}C_{14}T_{15}A_{16}T_{17} \; 5'$$

where for convenience we have numbered the base pairs from left to right; i.e., T_1 is the first (5′) base of the upper strand, A_1 the last (3′) base of the lower strand. There are eight AT and nine GC base pairs. The variety of magnetic environments makes possible the resolution of many of the individual base and sugar protons. Figure 1 shows a region of the NOESY spectrum containing cross-peaks between thymidine H6 protons and H3′ protons. Each of the thymidine H6 protons in this region cross-relaxes with two H3′ protons: a strong NOE with H3′ of its own sugar and a weaker NOE with that of its 5′ neighbor.[14] Thus, the sequential assignments of the base protons can be extended to assign the H3′ resonances of their sugar.

Figure 2 shows a region of the NOESY spectrum containing strong cross-peaks between the H1′ and H4′ sugar resonances. Since the H1′ resonance at 5.758 ppm has been assigned to T_{16},[7] its cross-relaxation with an H4′ at 4.04 ppm identifies this resonance as H4′ of the same sugar from the distance relations in the B DNA duplex. Cross-peaks a–h are identified similarly, and their assignments are given in the figure caption. Several trends are

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(10) A major groove base proton, such as a pyrimidine H6, may relax with its own or 5′-flanking sugar. The closest neighbor of H6 among its own sugar protons is H2′ (1.94 Å in standard B DNA). At short mixing times (<100 ms) this direct NOE predominates. Spin diffusion among protons of the same sugar observed at longer mixing times gives rise to indirect effects between such a base proton and H1′, H2″, H3′, and H4′. Similarly, a base proton gains magnetic access to the 5′-flanking set of sugar protons via H2″ (2.55 Å from an H6) and H1′ (2.96 Å from H6), from which spin diffusion may occur involving H2′, H3′, and H4′.
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(13) The COSY experiment, which identifies *J*-connected spin systems, provide a stringent consistency check for the assignment of the sugar protons. Resonances assigned to the same sugar must exhibit the appropriate pattern of *J* connectivities. The H2′ of T_{16}, for example, is connected to an H3′ resonance at 4.69 ppm, which was assigned above to the same sugar. The H3′ of T_{16} is connected to an H4′ resonance at 4.04 ppm, which was also assigned above to that sugar (data not shown).
(14) For example, the thymidine H6 resonance at 6.984 ppm, assigned to T_{13} by the sequential method,[7] cross-relaxes with its own H3′ at 4.74 ppm and, to a lesser extent, with the H3′ of G_{12} at 4.79 ppm. Similarly, the H8 proton of G_{12} at 7.512 ppm cross-relaxes with its own H3′ at 4.79 ppm and, to a lesser extent, with the H3′ of G_{11} at 4.85 ppm (data not shown).

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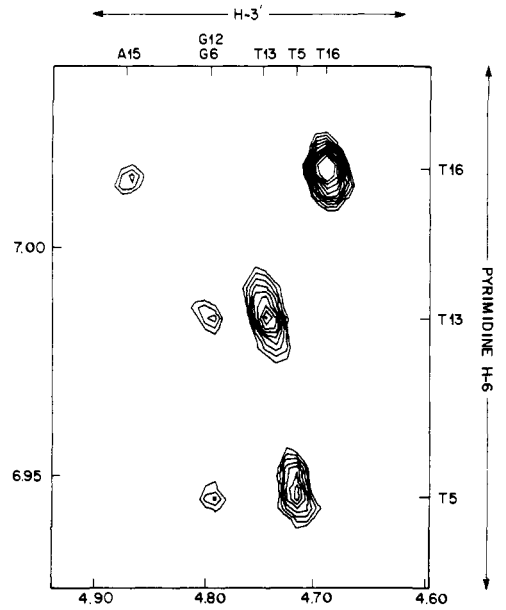

Figure 1. Portion of 2D NOESY spectrum showing indirect NOE's between thymidine H6 protons and H3′ protons. The characteristic geometry of B DNA implies that the larger effect is seen with the H3′ of its own sugar, the smaller effect with the H3′ of the 5′-flanking sugar. The oligonucleotide was made 6 mM in 200 mM KCl, 50 mM potassium phosphate (pD 7.4), 1 mM EDTA, and 1 mM sodium azide. The 2D experiment was a modification of the pure phase method of States et al.[3,7] and performed at 30 °C. The mixing time was 300 ms. 4096 points were sampled over 5000-Hz sweep width in t_2. After Fourier transformation on the fly, 1024 points containing the aromatic region were extracted and stored. 512 T_1 values were obtained and zero filled to 1024. A mild convolution difference with parameters GM2, EM100, and 0.9 was applied.

observed in this region of the NOESY spectrum. The H4′ resonances from pyrimidines (3.90–4.15 ppm) occur upfield to those from purines (4.22–4.36 ppm), with the exception of the terminal adenosines (A_1 and A_{17}), whose sugars may be in a different configuration.[7] As to the H1′ resonances, those from cytosines tend to be upfield to those from thymidines; likewise, the H1′ resonances from guanosines are upfield to those from adenosines.[7] Thus the two-dimensional H4′–H1′ region of the NOESY spectrum roughly partitions into four subsections corresponding to the four bases.[15]

O_L1 is the first complete operator DNA sequence for which H3′ and H4′ assignments have been made.[16] Since these protons lie on the outer surface of the sugar–phosphate backbone, they may be involved in ligand binding. In addition, H3′ assignments may be used to assign the scalar-coupled ^{31}P resonances by one-dimensional[17] and two-dimensional heteronuclear correlated methods.[5] These ^{31}P resonances are known to be sensitive to DNA conformation and interactions.[18-21] For these reasons we anticipate that this method will be of considerable utility in understanding the solution structure and dynamics of biologically important oligonucleotides such as O_L1.

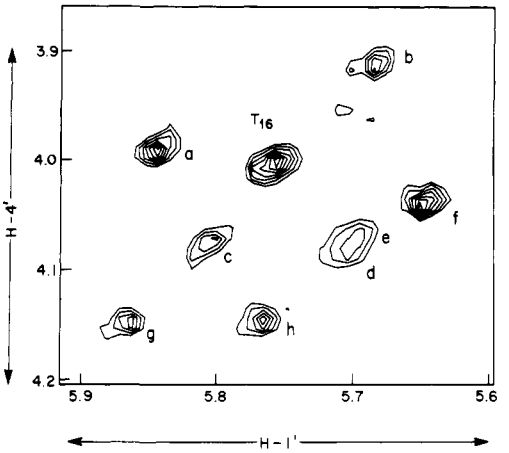

Figure 2. Portion of 2D NOESY spectrum showing indirect NOE's between pyrimidine H1′ and H4′ sugar protons. The conditions were the same as in Figure 1. H1′ assignments, obtained by the sequential method,[7] thus extend to the H4′ protons. The assignments shown are (a) T_2, (b) T_1 and T_{17}, (c) C_9, (d) C_6, (e) C_{12}, (f) T_{13} and T_5, (g) T_{15}, and (h) C_3. In the experiment the mixing time was 350 ms. Resolution enhancement was achieved in both dimensions by convolution difference with parameters 2, 20, and 1.

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(15) In addition to the strong NOE's observed between the H1′ and H4′ protons of the same sugar, weak cross-relaxation is observed between the H1′ of one sugar and the H4′ proton of the 3′-neighboring sugar on the same strand. Such NOE's, which may be used as consistency checks, illustrate that each sugar is not rigorously an independent relaxation sink, but only nearly so. The H1′ of A_{13}, for example, weakly cross-relaxes with an H4′ at 4.06 ppm, confirming the assignment of the latter to C_{12}, its 3′ neighbor. In standard B DNA, the distance from H1′ to the H4′ of the same sugar is 3.6 Å whereas the distance to the H4′ of the 3′-neighboring sugar is 4.2 Å. The strength of the latter NOE is therefore expected to be ≈40% of the former. In the single-crystal studies of Dickerson and Drew[9] the mean distances are 2.9 and 4.7 Å, implying an intensity ratio of 17:1. Although individual distances vary by as much as 0.9 Å relative to the mean value, the H1′ is always closer to its own H4′.

(16) The H3′ and H4′ resonances have been completely assigned with the exception of C_6 and G_{10}; the H3′ assignments of these bases are unclear due to limited resolution (manuscript in preparation).

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Synthesis and Crystallographic Characterization of an Unsolvated, Monomeric Bis(pentamethylcyclopentadienyl) Organolanthanide Complex, $(C_5Me_5)_2Sm$[1,2]

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The 1968 discovery of uranocene, $U(C_8H_8)_2$,[4] and the subsequent synthesis of the related lanthanide complexes,[5] $Ln(C_8H_8)_2^-$, demonstrated that eight-carbon rings rather than five-carbon rings were the appropriate size to form bis(ring) sandwich complexes of the f elements. This is quite reasonable given the strong preference of these large metals for high coordination numbers and typically 8–10 ligand electron pairs.[6,7] Bis(cyclopentadienyl)

(1) Dedicated to the memory of Professor Earl L. Muetterties, whose untimely death occurred on the date this structure was solved, Jan 12, 1984.
(2) Presented in part at the Industrial Associates Conference on Recent Trends in Heterogeneous and Homogeneous Catalysis, California Institute of Technology, March 21–23, 1984.
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