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Sequence-Specific Recognition of DNA: Assignment of Nonexchangeable Proton Resonances in the Consensus Pribnow Promoter DNA Sequence by Two-Dimensional NMR†

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ABSTRACT: The resonances of most of the nonexchangeable protons of both + and − strands of the consensus Pribnow dodecamer d(CGTTATAATGCG) have been assigned by two-dimensional nuclear magnetic resonance methods. Application of the two-dimensional nuclear Overhauser effect (NOESY) sequential connectivity method, combined with two-dimensional autocorrelated (COSY) spectra to reveal scalar-coupled protons, results in assignment of virtually all of the base and sugar protons, except the sugar C5 protons which are inadequately resolved. Analysis of the nuclear Overhauser data indicates that the helix assumes a fairly uniform B form conformation.

The recent improvements in both DNA synthesis and NMR[1] methods have allowed studies of nucleic acid structure in solution to be undertaken with the possibility of revealing detailed informtion on the sequence dependence of structure and dynamics. The first sequences that we have decided to examine by these methods are those involved in the regulation of the expression of DNA. Proteins interact with these sequences, i.e., promoters and operators, in a highly sequence-specific manner to either enhance or abolish the expression of specific gene loci in the adjacent DNA. Crystallographic studies have been carried out on several specific DNA binding proteins (Anderson et al., 1981; Pabo & Lewis, 1981; McKay & Steitz, 1981) and, in the case of the cro repressor from phage λ, have given rise to the first detailed model for the interaction of a regulatory protein with its cognate DNA (Ohlendorf et al., 1982). However, since no regulatory DNA sequences and only a few DNA binding proteins have been crystallized, it will certainly be of continuing interest to examine these nucleic acids and proteins in solution, and NMR is perhaps the only method available that can provide reasonably high-resolution structural information as well as dynamic information about such structures in solution. NMR evidence has shown that the conformation of DNA observed in crystals need not be preserved in solution (Reid et al., 1983a), thus providing additional impetus for the examination of solution structures of nucleic acids. In the present work, we report the proton assignments for, and preliminary structural analysis of, the consensus Pribnow promoter sequence d(CGTTATAATGCG). As described in the preceding paper (Chou et al., 1983), this sequence, or a close analogue, is involved in the binding of RNA polymerase prior to transcribing the DNA into the mRNA copy from which protein synthesis takes place (Rosenberg & Court, 1979; Siebenlist et al., 1980; Hawley & McClure, 1983).

The development of two-dimensional NMR methods has made possible the systematic assignment of resonances in many moderately large biopolymers. The application of 2D NMR to proteins has been described in detail by Wuthrich and co-workers (Wuthrich et al., 1982; Billeter et al., 1982; Wagner & Wuthrich, 1982), and more recently, these techniques have been applied to nucleic acids (Feigon et al., 1982; Pardi et al., 1983; Scheek et al., 1983; Hare et al., 1983). Other workers have used one-dimensional methods to achieve partial assignments of DNA spectra (Reid et al., 1983a,b; Sanderson et al., 1983; Kan et al., 1982). In 2D NMR, use is made of both through-bond and through-space couplings to identify groups of protons in bases and sugars of the nucleic acid and to establish which groups are neighbors in the primary sequence. Once the assignments of the resonances to particular protons have been made, one can then proceed to the study of DNA structure and DNA–ligand interactions. This is

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[1] Abbreviations: NMR, nuclear magnetic resonance; 2D NMR, two-dimensional NMR; NOESY, two-dimensional nuclear Overhauser effect; COSY, two-dimensional autocorrelated; DSS, 4,4-dimethyl-4-silapentane-1-sulfonate.

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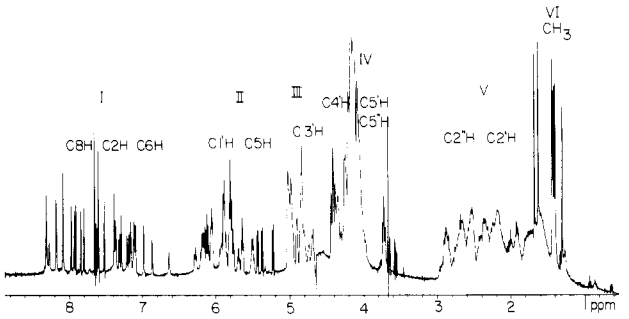

FIGURE 1: Proton NMR spectrum (500 MHz) of the consensus Pribnow dodecamer CGTTATAATGCG in D_2O solution at 37 °C. A total of 128 scans was averaged, and resolution was enhanced with a Lorentzian to Gaussian transformation. The various spectral regions are labeled with Roman numerals, and the types of protons giving rise to resonances in each region are indicated.

possible because the NOEs, which are used in the assignment of resonances, also provide direct structural information in the form of interatomic distances, which can be used to analyse the average structure in solution.

Materials and Methods

DNA Synthesis. DNA synthesis was carried out by the solid-phase phosphite triester method, as described previously (see accompanying paper). The samples used for two-dimensional NMR work were prepared by repeated lyophilization from D_2O solution. For the final solution, about 10 mg of DNA was dissolved in 0.4 mL of 99.996% D_2O (Stohler Isotope Chemicals). The sample also contained 10 mM phosphate buffer, pH 7.0, and 100 mM NaCl.

NMR Spectroscopy. NMR spectra were taken on a Bruker WM500 spectrometer. COSY and NOESY spectra were taken at 37 °C by using 1024 complex points in t_2 and about 350 points in t_1, with appropriate phase cycling to achieve quadrature detection in both domains (Macura et al., 1981). For COSY spectra, 96 scans were averaged at each t_1 value, with a recycle delay of 1.5 s in addition to the aquisition time of 0.23 s. For NOESY spectra, 128 scans were averaged, the recycle delay was 2.2 s, and a mixing time of 300 ms was used. The mixing time was stochastically varied by 10% to suppress cross peaks arising from coherence transfer (Macura et al., 1981). The data were written onto magnetic tape, transferred to a VAX 11/780 computer, and processed and plotted using FT2D software we have written (D. R. Hare, unpublished results). A skewed sine-bell apodization was applied after which the data were zero filled to achieve a final matrix of 1024 × 1024 points, giving a final digital resolution of 4.3 Hz in each dimension. Spectra were symmetrized by the method of Baumann et al. (1981). The residual water signal was supressed by weak continuous irradiation except during the acquisition period.

Results and Discussion

The one-dimensional spectrum of nonexchangeable protons in the consensus Pribnow promoter sequence embedded in two GC pairs at each end is shown in Figure 1. The grouping of peaks according to chemical type is typical for DNA and is shown above the spectrum; there are six reasonably discrete regions of the spectrum that have been labeled I–VI. For this asymmetric dodecamer there are 36 base protons, 7 methyl groups, and 168 sugar protons that contribute to the spectrum with regions IV (72 protons) and V (48 protons) being the most crowded. The principles of the two-dimensional assignment method for helical DNA have been described in detail elsewhere (Hare et al., 1983) and, hence, will only be

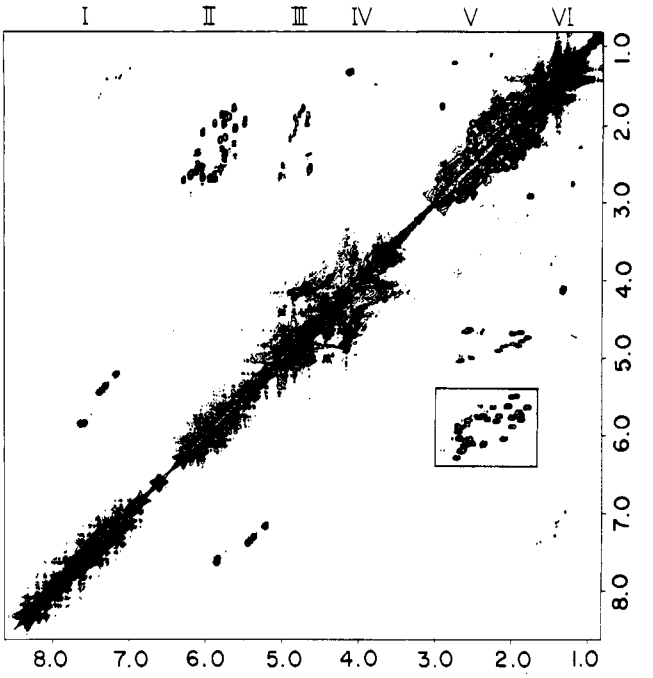

FIGURE 2: Contour plot of the COSY spectrum of the Pribnow dodecamer in D_2O solution at 37 °C. Regions are labeled as in Figure 1. Cross peaks from DSS and from a contaminant are observed in the V–VI and IV–VI regions. These impurities do not give rise to cross peaks in the NOESY spectrum. The box in the II–V region is shown expanded in Figure 8.

briefly reviewed here, except in the cases where particular problems occur. The COSY spectrum makes use of scalar couplings to group together protons on a given residue. Each cytosine gives rise to a pair of coupled peaks (C6-H and C5-H), which generate an off-diagonal cross peak in the I–II region, while each thymine gives rise to a methyl resonance and a C6-H, which are weakly coupled through four bonds and generate a cross peak in the I–VI region. Adenine and guanine do not have any pairs of coupled protons and, hence, have no off-diagonal peaks in the COSY spectrum. The sugar protons can also be grouped through their couplings. Each deoxyribose ring will give rise to set of coupled protons C1′H–C2′H,C2″H–C3′H–C4′H–C5′H,C5″H.

The COSY spectrum of the Pribnow dodecamer is shown in contour plot form in Figure 2. While the coupling patterns identify all of the protons on a particular base or sugar, they do not provide any information about the position of that base or sugar in the sequence. This complementary information comes from the cross-relaxation patterns in the NOESY spectrum. Since NOEs arise from spatial proximity rather than through-bond connectivity, they can be used to identify the nearby protons of the neighboring base and sugar. The type of base in the adjacent position can then be identified through its coupling pattern in the COSY spectrum, and this type of sequential identification allows absolute assignments to be made by following the known primary sequence. NOEs occur first to the nearest-neighbor protons and then for long mixing times may spread in a domino-like effect (generally refered to as spin diffusion) to more distant protons. Because the NOEs from the closest neighbors must develop *first*, the fact that all the observed cross peaks can be accounted for in terms of direct NOEs to the expected nearest neighbors and no additional cross peaks are observed indicates the absence of significant spin diffusion.

A contour plot of the NOESY spectrum of the consensus Pribnow sequence is presented in Figure 3. In many cases, several NOEs to neighboring residues are observed, thus

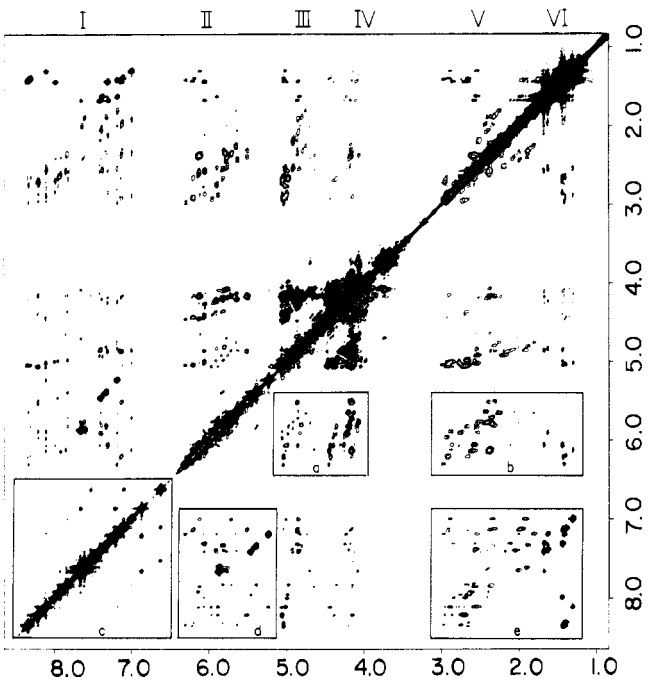

FIGURE 3: Contour plot of the NOESY spectrum under conditions identical with those in Figure 2. Various regions that are shown expanded in other figures are indicated by letters: (a) Figure 9; (b) Figure 8; (c) Figure 10; (d) Figure 6; (e) Figure 7.

providing redundant information that is very useful for checking the consistency of the sequential assignments as they progress. The principal NOEs used to connect adjacent residues are those between the aromatic protons and C1′ protons. In B DNA, a given aromatic proton gives a NOE not only to its own C1′-H but also to the C1′-H of the neighboring sugar on the 5′-side (Hare et al., 1983; Scheek et al., 1983)—see Figure 4. Additional NOEs from the aromatic proton (C6-H or C8-H) to the C2″-H of the 5′-neighbor and to the aromatic proton of the 3′-neighbor or the 5′-neighbor (depending on whether the adjacent base is a pyrimidine or a purine) are also valuable for assigning peaks when degeneracies occur for some of the aromatic protons and confirm assignments made through the C1′-H connection—see Figure 4. The basis for the assignments can be schematically represented in a diagram using d_1, d_2, and d_3 to represent the aromatic–aromatic, aromatic–C1′-H, and aromatic–C2″-H NOEs, respectively. The different types of interresidue NOEs observed for the consensus Pribnow sequence are schematically presented in Figure 5 for each DNA strand.

Some particular features of the assignment procedure for this asymmetric dodecamer are worth noting, since they did not arise in the simpler symmetrical sequence that we have described previously (Hare et al., 1983). The two strands of the helix must be treated separately, since there are no proton–proton distances below 5 Å between strands. In addition to the group of sugar protons and the C5, C6, and C8 base protons, there is an additional group of aromatic protons in the center of the helix, the adenine C2-Hs, which are neither scalar- nor dipolar-coupled to either of the other proton groups. Since there are seven consecutive AT base pairs in this promoter sequence, there is a group of seven C2-H protons in region I that should show cross peaks only to each other in D_2O solutions. These adenine C2-Hs show NOEs to imino protons in H_2O solution, as described in the preceding paper.

The region of the NOESY spectrum that is most useful to begin the sequential assignments is the aromatic to C1′ region (box d in Figure 3), shown in expanded form in Figure 6. The COSY cross peaks in the I–II region (aromatic C6-H–aromatic C5-H) and in the I–VI region (aromatic C6-H–methyl) identify the aromatic protons of the five cytosines and the seven thymines in the sequence. The remaining peaks in region I give no COSY cross peaks and are derived from adenine and guanine protons. The chemical shifts of the individual C1′-H resonances can be determined by observing their couplings to the C2′ and C2″ region (Figure 2 and box b in Figure 3). Knowledge of the distribution of these peaks is only important when chemical shift degeneracies occur, in which case elimination can be used to aid in the assignment procedure. For the Pribnow dodecamer, all of the possible cross peaks in the I–II region were observed, though partial overlap occurs for

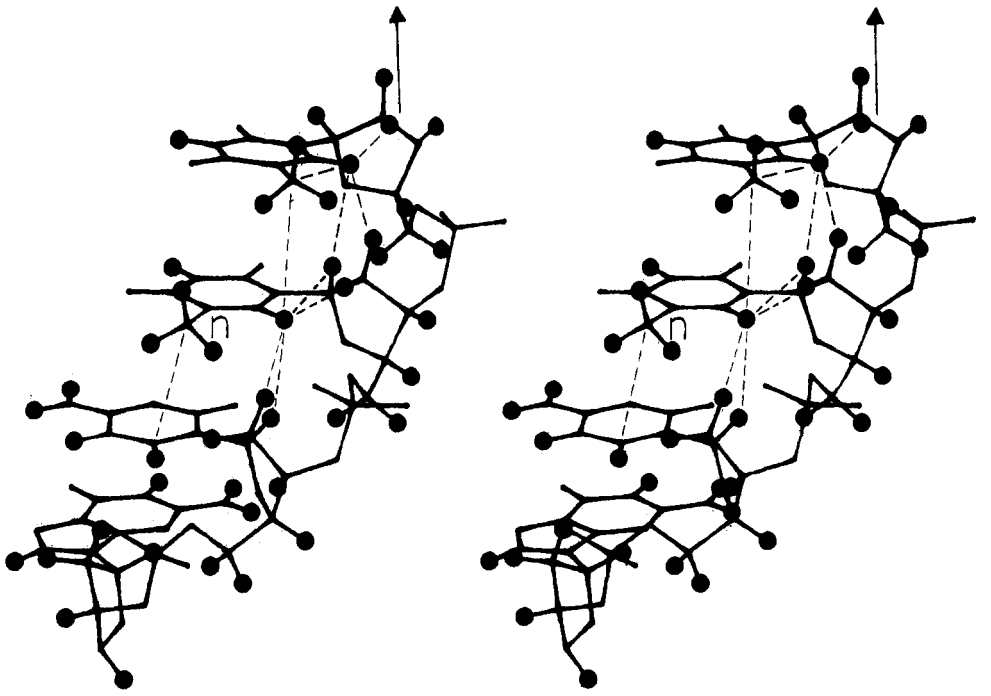

FIGURE 4: Stereo view showing the spatial proximity and NOE connectivity network between protons on adjacent residues in the idealized sequence dGCTT. The interior T residue is arbitrarily chosen as residue *n* and the 5′ to 3′ direction is from bottom to top.

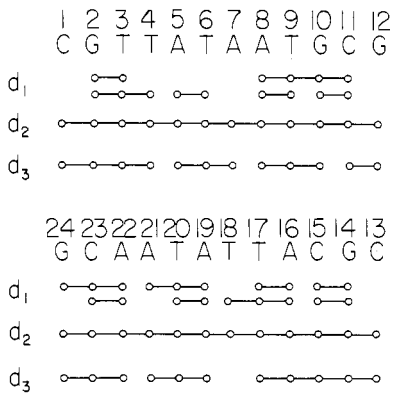

FIGURE 5: Schematic representation of the NOEs observed and used for assignments for the consensus Pribnow sequence. The terms d_1, d_2, and d_3 represent NOEs from the aromatic proton to the (a) aromatic, (b) C1′, and (c) C2″ protons of the neighboring residue, respectively. Residues for which such NOEs were observed are connected with a solid bar on the corresponding line. For d_1, the upper line represents NOEs from a pyrimidine C5 proton to a neighboring C6 or C8, while the lower line represents NOES from C6 to C6, C6 to C8, or C8 to C8 protons, depending on the sequence.

some of them. The chemical shifts of the two most downfield-shifted cytosine C6-H resonances, by analogy with previous studies on other DNAs, suggest that they arise from the two 5′-terminal cytosines in the sequence, one on each strand. By beginning with the more downfield of these, the sequence of interresidue aromatic to C1′-H cross peaks can be followed along the chain through G-12 and is shown traced in Figure 6A. It should be emphasized that the ad hoc assumption concerning the chemical shift of the terminal cytosine C6-H is not at all crucial to the assignment procedure; one can start with any aromatic proton and, after two or three steps, establish where one is in the chain merely by consulting the sequence. The C1′-H resonances of G-2 and T-3 are almost degenerate; however, the identification of the neighboring T-3 and T-4 via the methyl to aromatic region (shown expanded in Figure 7), together with the lack of a second cross peak for the T-3 C6-H resonance, leads to their unambiguous assignment. Similarly, the C1′-H protons of G-10 and C-11 are almost degenerate, but again, the lack of other cross peaks for the C-11 C6-H resonance and the NOE from the G-10 C8-H to the C-11 C5-H allow these assignments to also be made unambiguously. The identification of the C-1 and C-13 aromatic protons must be carried out by using the C2′,C2″ to aromatic region of the NOESY spectrum because their C1′ protons have almost identical chemical shifts and their cross peaks to the aromatics are not well resolved, whereas their C2′ protons are significantly different in chemical shift. The other strand of the helix, i.e., C-13 to G-24, is straightforward to assign and is traced in Figure 6B. The other pair of adjacent thymidines in this strand, T-17 and T-18, again provide a convenient check in the aromatic to methyl region by which assignments can be confirmed. The chemical shifts of aromatic protons A-7 and A-21, and likewise those of A-8 and A-22, are almost superimposed. The assignment of A-7 and A-21 is based upon the very small chemical shift difference in the aromatic protons of A-8 and A-22, allowing the cross peaks to A-7 and A-21 to be distinguished. Even if these assignments are reversed, the remainder of each strand can be correctly assigned since the two strands differ in primary sequence. The specific assignment of all of the cytosine C5 protons and the thymine methyls is immediately evident from the COSY cross peaks once their particular C6 protons have been assigned.

Assignment of the C2′ and C2″ protons can now be made from the assigned C1′ and aromatic protons. Each C1′ proton

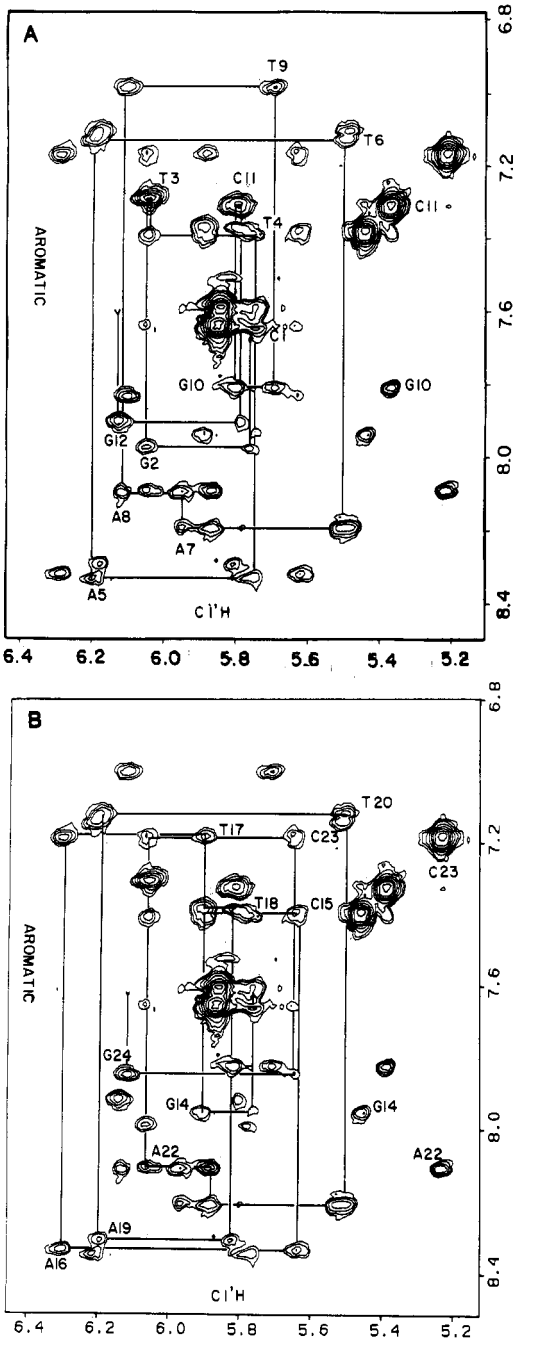

FIGURE 6: Expansion of box d of Figure 3 in the NOESY spectrum (region I–II). (A) Cross peaks for the + strand (residues 1–12) are connected in a sequential pattern, with each cross peak between an aromatic and the C1′ proton on the same residue identified by that residue. (B) The same region tracing the − strand (residues 13–24) in the same fashion. The large cross peaks in the 5.2–5.45 ppm region arise from NOEs between cytosine C5 and C6 protons on the some residue, while the smaller peaks arise from purine C8 to neighboring cytosine C5 proton NOEs.

shows a strong NOE to the C2″-H and a weaker NOE to the C2′ proton on the same sugar. When degeneracies occur in the C1′ region, the correct C2′-H and C2″-H can be identified through their NOEs to the aromatic protons on their own base, and by their weaker NOEs to the neighboring base on the 3′-side. The NOESY spectra of the C1′-H to C2′-H,C2″-H region and of the C2′-H,C2″-H to aromatic region are shown in Figures 8 and 7, respectively.

At this stage, the C3′ protons can now be assigned by using scalar couplings to their C2′ and C2″ protons together with direct NOEs from the C1′ protons and from the aromatic protons. The large number of C3′ resonances occur over a

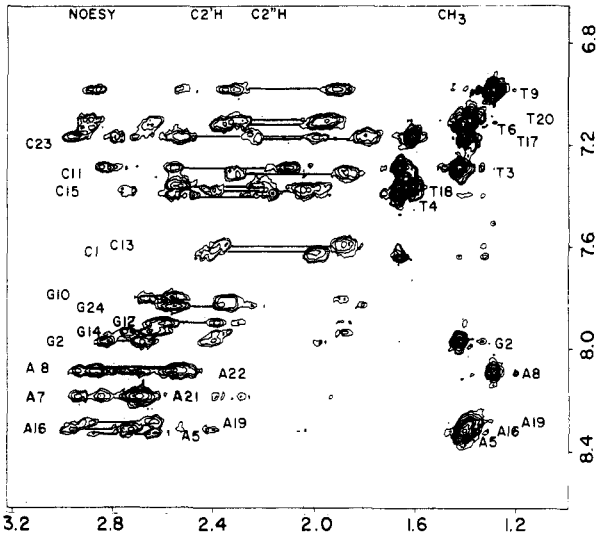

FIGURE 7: Expansion of box e (regions I–V and VI) of the NOESY spectrum show in Figure 3. Horizontal bars connect the aromatic to C2′ and C2″ cross peaks of the same residue and are identified with that residue. The remaining peaks in the I–V region represent NOEs to C2′ and C2″ protons of residues on the 5′-side. The peaks in the I–VI region are aromatic to methyl NOEs.

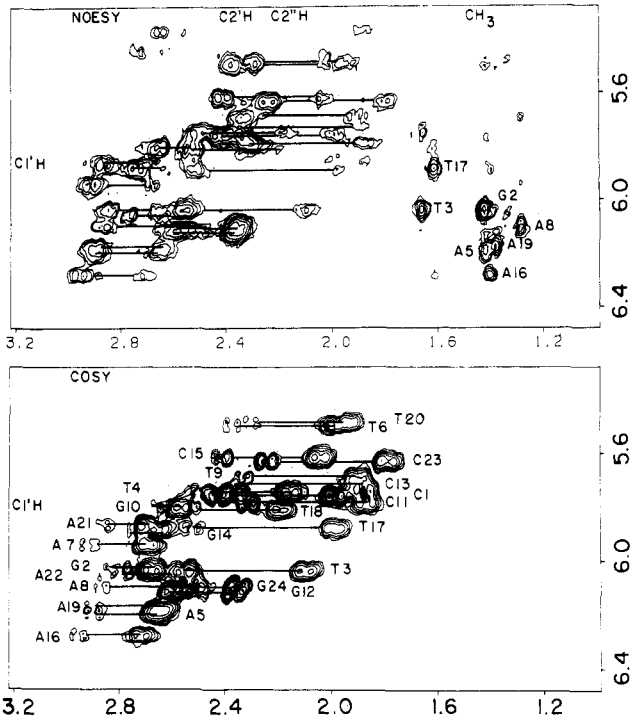

FIGURE 8: Expansion of box b (regions II–V and VI) of the NOESY spectrum of Figure 3 and the box of the COSY spectrum of Figure 2. The differences in cross-peak intensities in the COSY or NOESY spectra allow the C2′ protons to be distinguished from the C2″ protons. In the NOESY spectrum, cross peaks between thymine methyls and the C1′ protons on the residue to the 5′-side are also observed.

rather limited chemical shift range so that, although the position of the C3′ protons can be identified and the cross peaks involving the C3′ protons can be labeled with the two protons that are coupled, the true resolution in region III is poor. The assignments of the C4′ protons are accomplished in an exactly analogous fashion, with the C3′ to C4′ couplings in the COSY spectrum used to check the direct C1′-H to C4′-H NOEs. Again, the spectral region in which the C4′ resonances occur is quite narrow, and the resolution is limited. As we previously pointed out during assignment of the simpler symmetrical

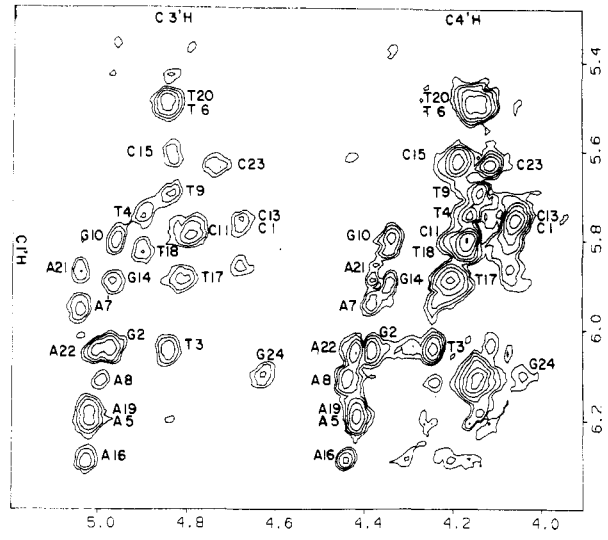

FIGURE 9: Expansion of box a (regions II–III and IV) of the NOESY spectrum in Figure 3. After the C1′ protons have been assigned from box c (Figure 3), this region allows assignment of the C3′ and C4′ protons, and these peaks have been labeled with their residue number. The III–V and III–VI regions allow an independent check of most of the C3′ assignments. Peaks not identified in the C1′ to C4′ region probably arise from C1′ to C5′ or C5″ NOEs. The C1′ to C3′ cross peaks for guanine-12 were not observed.

CGCGAATTCGCG sequence (Hare et al., 1983), the resolution of the C4′ protons from the C5′ and C5″ protons is almost nonexistent, except for the ends of the helix. For this reason, as was true for the symmetrical dodecamer, the 5′ and 5″ protons cannot be assigned. The expansion of the NOESY spectrum used for assigning the C4′ protons and the C3′ protons from their C1′ protons is shown in Figure 9.

In the aromatic region of the NOESY spectrum there are a number of cross peaks among resonances that show no other NOEs to sugar protons. These arise from adenine C2-Hs in the center of the helix. From these cross peaks the position of six of the seven C2-H resonances can be identified, and two of the C2-Hs are clearly sandwiched between adjacent adenine C2-Hs. These have been previously assigned by their NOEs from imino protons in the preceding paper, and the NOEs observed here are completely consistent with these assignments. The reason for there being only four C2-H–C2-H cross peaks is that the cross peaks from the pairs of adenines 5 and 6 and 8 and 9 are too close to the diagonal to observe; i.e., their chemical shifts are too similar. In addition to the relatively strong cross peaks between the C2-H protons, a number of other weaker NOEs are observed between aromatic protons in region I. These arise from C6-H and C8-H protons of adjacent bases. Since the C6-H and C8-H protons have relatively similar chemical shifts, the direct NOE cross peaks from C6-H to C6-H of adjacent pyrimidines and from C8-H to C8-H of adjacent purines are both weak and close to the diagonal and, hence, cannot be reliably observed. An expansion of the aromatic to aromatic NOE region is shown in Figure 10. The chemical shifts of all of the assigned protons are summarized in Table I.

The NOEs observed in the Pribnow dodecamer are, in general, consistent with a normal B form DNA structure in solution, as was the case for the symmetrical dodecamer described previously (Hare et al., 1983), in that the intraresidue and interresidue through-space connectivities predicted for B DNA are observable and NOE intensities agree qualitatively with expected distances. However, variations in the cross-peak intensities in the C1′ to aromatic region may represent slight differences in the local structure of the sugar residues. Un-

Table I: Assignment of the Consensus Pribnow Sequence

			1 C- G- 24	2 G- C- 23	3 T- A- 22	4 T- A- 21	5 A- T- 20	6 T- A- 19	7 A- T- 18	8 A- T- 17	9 T- A- 16	10 G- C- 15	11 C- G- 14	12 G C 13	
residue	C8-H	C6-H	C5-H		C2-H		CH_3		C1′-H	C2″-H	C2′-H	C3′-H	C4′-H		
C-1		7.63	5.84						5.75	2.37	1.88	4.67	4.06		
G-2	7.98								6.03	2.84	2.67	4.97	4.37		
T-3		7.29					1.41		6.04	2.55	2.10	4.84	4.24		
T-4		7.39					1.66		5.75	2.43	2.16	4.84	4.16		
A-5	8.33				7.19				6.18	2.91	2.65	5.02	4.42		
T-6		7.13					1.41		5.49	2.37	2.01	4.84	4.14		
A-7	8.18				6.60				5.94	2.91	2.69	5.03	4.37		
A-8	8.10				7.50				6.10	2.85	2.53	4.99	4.43		
T-9		6.98					1.28		5.69	2.31	1.90	4.84	4.13		
G-10	7.81								5.81	2.64	2.57	4.96	4.34		
C-11		7.31	5.37						5.78	2.31	1.88	4.79	4.18		
G-12	7.90								6.11	2.57	2.37	*a*	*a*		
C-13		7.58	5.85						5.74	2.43	1.88	4.67	4.06		
G-14	7.93								5.88	2.74	2.62	4.97	4.34		
C-15		7.38	5.44						5.61	2.42	2.05	4.83	4.18		
A-16	8.32				7.58				6.28	2.97	2.72	5.02	4.43		
T-17		7.16					1.40		5.88	2.49	1.98	4.80	4.20		
T-18		7.37					1.60		5.80	2.55	2.19	4.90	4.18		
A-19	8.29				7.08				6.17	2.90	2.63	5.02	4.42		
T-20		7.10					1.37		5.47	2.31	1.94	4.84	4.14		
A-21	8.19				6.84				5.86	2.84	2.68	5.03	4.37		
A-22	8.09				7.63				6.04	2.76	2.64	5.00	4.42		
C-23		7.16	5.21						5.63	2.25	1.79	4.73	4.08		
G-24	7.83								6.10	2.52	2.35	4.58	4.04		

[a] Not assigned; C1′ to C3′ cross peak not observed.

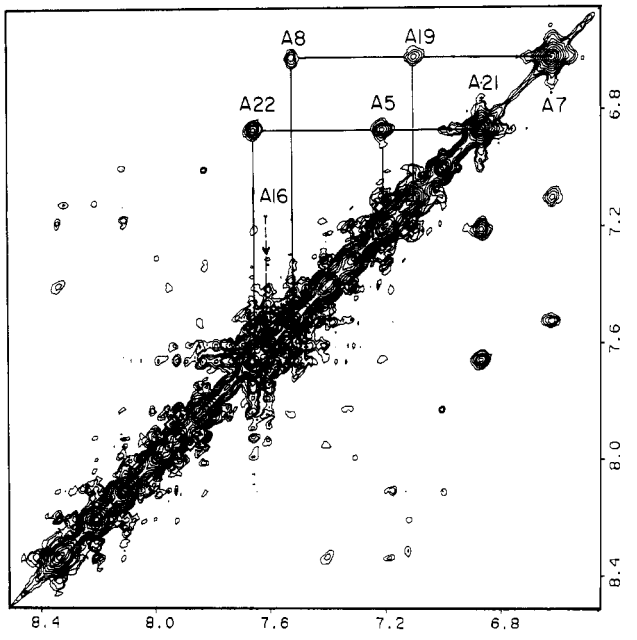

FIGURE 10: Expansion of box c of the NOESY spectrum of Figure 3. The cross peaks between the adenine C2 protons of the seven consecutive AT base pairs are connected. The other small cross peaks arise from C6-H to C8-H NOEs between neighboring bases.

fortunately, the cross-peak intensities are not a simple function of the distances between the protons; a more detailed analysis, which is required to obtain more precise structural information, is presently under way. Other sequences that we have studied (unpublished results) show larger deviations in structure from the B form than does the Pribnow sequence. In the Pribnow dodecamer, the most upfield of the adenine C2-H resonances are broader than the majority of other resonances. This may indicate that there are conformational fluctuations that affect the chemical shift of these protons which are located on base pairs 4–7 in the helix. The nature of these fluctuations cannot be determined from the measurements described here since their rates are near the fast-exchange limit. It is possible that these fluctuations are responsible for exchange of the imino protons with solvent, as described in the preceding paper. The intensities of the NOEs observed between pairs of adenine C2 protons are also unequal, suggesting a nonuniform structure. Although different NOE intensities might be expected between AA and AT sequences, even the two AA sequences show unequal NOEs, as do the two pairs of adjacent AT residues. A likely explanation for this observation is "propeller twisting" of the base pairs. If we assume that the NOE buildup is still in its linear phase during the 0.3-s mixing time used, then the ratios of the NOEs should be proportional to the sixth power of the corresponding distances. The distances can be calibrated by using the cytosine C5-H to C6-H cross peaks as a reference NOE intensity since these two protons are fixed and separated by a known distance. By use of this crude approach, the variations in the C2-H–C2-H distances appear to be approximately 0.5 Å. The interpretation of these distances in terms of particular twist angles for the base pairs requires further refinement, and we are working toward this goal. Such differences have also been observed for a symmeterical TATA sequence and were analyzed in terms of propeller twist (Patel et al., 1983).

In the symmetrical CGCGAATTCGCG dodecamer studied previously (Hare et al., 1983), significantly different geometries were seen for the sugars at the ends of the helix. For the consensus Pribnow sequence, these end effects also seem to be present, but to a much smaller degree. The aromatic to C1′-H NOEs are observed for all sugars, including the terminal ones. The unusually large guanine-2 C8-H to cytosine-1 C2′-H NOE seen in the symmetrical sequence above is not observed in the Pribnow sequence, although the end two base pairs are identical. Whether these differences arise from sequence effects or from differences in experimental conditions

(i.e., ionic strength) is as yet unclear.

Registry No. d(CGTTATAATGCG)·d(CGCATTATAACG), 89144-59-2.

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Cobalt–Bleomycins and Deoxyribonucleic Acid: Sequence-Dependent Interactions, Action Spectrum for Nicking, and Indifference to Oxygen†

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ABSTRACT: Light-induced strand scission of DNA by cobalt–bleomycins is more likely to occur at certain base sequences than others. By use of ^{32}P-end-labeled DNA restriction fragments as the substrates for cleavage, products have been analyzed on high-resolution polyacrylamide gels and compared to those produced by iron–bleomycin. The results indicate that the sites of damage to DNA are similar in both cases: pyrimidine residues located at the 3′ side of a guanine are preferentially attacked. Consistent with the observed nicking specificity, interactions between cobalt–bleomycin and guanine residues in the trinucleotide sequence GGT are revealed in a dimethyl sulfate methylation experiment. The action spectrum for the light-induced DNA cleavage reaction correlates with the absorption spectrum of cobalt–bleomycin in the wavelength range between 330 and 450 nm. In contrast to iron–bleomycin, the extent of DNA degradation by light-activated cobalt–bleomycins appears to be indifferent to the concentration of dissolved oxygen in the reaction medium, and little or no base propenal is produced. Bases (e.g., thymine) are released by both agents. Fluorescence quenching experiments show that apparent binding constants of cobalt–bleomycin complexes with DNA are in the 10^7 M^{-1} range in 25 mM tris(hydroxymethyl)aminomethane, pH 8, 1 mM NaCl, and 1 mM ethylenediaminetetraacetic acid at 25 °C.

Bleomycin (BLM)[1] is the name of a group of glycopeptide antibiotics used clinically in the treatment of cancer (Umezawa et al., 1966; Blum et al., 1973; Crooke & Bradner, 1976); the drug is thought to act in vivo by chemically degrading cellular DNA (Suzuki et al., 1969; Terasima et al., 1970).

Bleomycin forms complexes with transition metal ions, and its biological activity appears to require metal coordination (Sausville et al., 1976). Metallobleomycins thus formed are capable of inducing strand scission of DNA in vitro. For example, iron–bleomycin actively degrades isolated DNA in the presence of molecular oxygen and a variety of reducing agents (Sausville et al., 1976, 1978a,b; Lown & Sim, 1977). Cleavage of DNA by copper–bleomycin (Murugesan et al., 1982; Freedman et al., 1982; C.-H. Chang and C. F. Meares, unpublished data), and by manganese– and nickel–bleomycins as well (C.-H. Chang and C. F., Meares, unpublished data), may also occur under appropriate conditions.

Recently, we have found that cobalt(III)–bleomycins can cause strand scission of DNA in the presence of light (Chang & Meares, 1982). There are several different cobalt(III)–bleomycins, including an orange complex in which all six ligands to cobalt are supplied by bleomycin, a green complex

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[1] Abbreviations: BLM, bleomycin; Co(III)–BLM, a complex of cobalt(III) with bleomycin; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; bp, base pair.

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