Two-Dimensional Proton Nuclear Magnetic Resonance Investigation of the Synthetic Deoxyribonucleic Acid Decamer d(ATATCGATAT)$_2$†

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ABSTRACT: In this study two-dimensional NMR techniques (COSY and NOESY) have been used in conjunction with one-dimensional NMR results to complete the assignment of the proton NMR spectrum of the double-stranded DNA decamer, d(ATATCGATAT)$_2$, and to obtain qualitative information about numerous interproton distances in this molecule and some limited information about conformational dynamics. COSY and NOESY measurements have been combined to systematically assign many of the resonances from the H1′ and H2′,2″ sugar protons to specific nucleotides in the double helix. This method relies on the fact that sugar protons *within* a specific nucleotide are scalar coupled and that base protons (AH8, GH8, TH6, and CH6) in right-handed helices can interact simultaneously with their own H2′,2″ sugar protons and those of the adjacent (5′–3′) nucleotide attached to its 5′ side (i.e., XpA not ApX). A COSY experiment is used to identify sugar resonances within a residue whereas the NOESY experiment allows the neighboring sugar to be connected (linked). The CH5 and CH6 resonances in the spectrum can immediately be identified by the COSY experiment. The methyl protons of thymine residues exhibit strong through-space interbase interactions both with their own TH6 proton and with AH8 proton on the adjacent (5′–3′) adenine residue. These interactions are used both to make assignments of the spectra and to establish that the thymine methyl groups are in close proximity to the AH8 protons of adjacent adenine residues [Feigon, J., Wright, J. M., Leupin, W., Denny, W. A., & Kearns, D. R. (1982) *J. Am. Chem. Soc. 104*, 5540]. Interactions between the base protons (PuH8 and PyH6) and the H1′ sugar protons are quite weak and probably arise from second-order NOEs (via the 2′,2″ protons). The strong interactions between the base protons and the H2′,H2″ sugar protons indicate that the base protons are in close proximity to the 2′ sugar proton on the same nucleotide unit and only slightly further away from the 2″ sugar proton of the adjacent (5′–3′) nucleotide unit. This establishes that the nucleotides have an anti conformation of the bases relative to the sugar. The collection of inter- and intranucleotide interactions observed in the NOESY experiments places restrictions on the possible conformational state of this decamer. These results, in conjunction with the results of a set of 1D-NMR experiments, provide the foundation for a more rigorous determination of the structure of this molecule in solution.

Renewed interest in the structural properties of DNA, stimulated by the recent discovery of Z-DNA (Wang et al., 1979; Drew et al., 1980) and X-ray diffraction studies on other double-helical DNAs (Wing et al., 1980), has reemphasized the need for methods that will provide detailed structures of DNA molecules in solution. Spectroscopic methods previously used in solution-state investigations of DNA have clearly been inadequate; however, recent developments in nuclear magnetic resonance spectroscopy have provided new approaches to this long-standing problem. Specifically, a collection of one- and two-dimensional NMR relaxation techniques have been developed (Jeener, 1971; Freeman & Morris, 1978; Jeener et al., 1979; Macura et al., 1981) and applied to the examination of the structural and dynamic properties of moderately large macromolecules (Kumar et al., 1980a; Nagayama et al., 1979; Broido & Kearns, 1980, 1982). In several recent papers we have described the use of various one-dimensional relaxation techniques in studies of the structural properties of RNA and various double-stranded DNA helices (Early & Kearns, 1979; Early et al., 1980; Broido & Kearns, 1980, 1982; Kearns et al., 1981; Feigon et al., 1982a). In this paper, we discuss the application of two-dimensional (2D) NMR to an examination of a double-stranded DNA, d(ATATCGATAT)$_2$. A preliminary account of some of this work has been given elsewhere (Feigon et al., 1982a,b, 1983).

The virtue of the 2D-NOE technique is that it permits all major dipolar interproton interactions in a molecule to be detected in a single experiment. In DNA molecules, this allows one to identify those pairs of protons which are physically close together, and this information is useful both in making assignments and in determining conformation. Similarly, all scalar coupled protons in molecules can be efficiently identified in a single experiment by using two-dimensional *J* spectroscopy (Bax & Freeman, 1981). In DNA, all protons within a single sugar residue are coupled so 2D-*J*-correlated spectroscopy makes it possible, at least in theory, to identify all of the protons in a specific sugar.

In this paper we apply 2D-NOE and 2D-*J*-correlated spectroscopy to a study of the synthetic DNA decamer d(ATATCGATAT)$_2$. These two techniques have been used to make, or confirm, assignments of the various proton resonances in the spectra and to obtain qualitative information about the relative magnitudes of various inteproton distances in the molecule. This information provides important restrictions regarding the probable conformational state of d(ATATCGATAT)$_2$ in solution. These studies (Feigon et al., 1982a,b, 1983) establish the feasibility of the 2D-NMR

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methods and provide the necessary foundation for quantitative NMR studies of the conformational and dynamic properties of DNA molecules in solution.

Materials and Methods

Materials. The DNA decamer, d(ATATCGATAT)$_2$, was synthesized in this laboratory, as described elsewhere (Denny et al., 1982). Samples, dissolved in 10 mM sodium phosphate, pH 7.0, and 0.1 M NaCl, were contained in either a Wilmad 508 cp microcell (3 mM duplex, 120 μL) or a thin wall tube (2 mM duplex, 200 μL). The samples were repeatedly dried with N_2 gas and redissolved in 99.996% D_2O before use.

Methods. The experiments were conducted on a 360-MHz spectrometer which employed a 8.45-T Oxford magnet interfaced to a Nicolet 1180E computer and modified Varian HR-220 console as previously described (Wright et al., 1981). Some spectra were obtained on the regional 500-MHz spectrometer at the California Institute of Technology. Chemical shifts are relative to the chemical shift of water which had been carefully referenced to sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as a function of temperature.

2D-NOE Spectroscopy. The basic pulse sequence for the 2D-NOE (NOESY) experiments is (Jeener et al., 1979; Kumar et al., 1980)

$$[P_2\text{–}t_1\text{–}P_2\text{–}\tau_m\text{–}P_2\text{–}t_2\text{–}D_5]_n$$

where P_2 indicates a 90° pulse, t_1 is an evolution time during which the various magnetization components are frequency labeled, τ_m is a mixing time during which dipolar (or chemical exchange) interactions between protons are manifested as selective NOEs, t_2 is the time during which the signal is recorded, and D_5 is a delay time to allow the magnetizations to relax back to equilibrium. For a given experiment, τ_m remains fixed and t_1 is incremented by the dwell time (half the inverse spectral width). The resulting data matrix obtained (t_1, t_2) is double Fourier transformed to give a 2D spectrum in the frequency domain (f_1, f_2). Pure absorption phase spectra were obtained following the procedure described by States et al. (1982), but a phase cycling routine containing only 32 steps was used to cancel out components of transverse magnetization remaining after the second 90° pulse and to suppress axial ridges due to recovery of equilibrium magnetization during the mixing time. The phase cycling scheme used permits quadrature detection in both dimensions with the carrier located at the center of the spectrum. The resulting software program of 101 steps was reduced to 8 or 16 steps by modification of the Nicolet 293B pulse programmer (J. M. Wright, unpublished results). This phase cycling also suppresses undesirable J cross peaks arising from single- and double-quantum coherences but does not affect the zero quantum coherences (Macura et al., 1981; Kumar et al., 1981). For the relatively long mixing times used in most of the experiments shown here, the NOEs are expected to dominate over the coherences due to J coupling. Further experimental details are given in the figure captions and text.

2D-J-Correlated Spectroscopy. The basic pulse sequence for the 2D homonuclear J-correlated (COSY) experiment is (Aue et al., 1976)

$$[P_2\text{–}t_1\text{–}P_2\text{–}t_2\text{–}D_5]_n$$

where P_2 indicates a 90° pulse, t_1 is the evolution period between the two pulses during which the various magnetization components are labeled with their characteristic frequencies, t_2 is the time during which the signal is recorded, and D_5 is the delay between successive acquisitions to allow for spin–lattice relaxation. The 2D spectrum obtained after data processing looks similar to the 2D NOE, except in this case

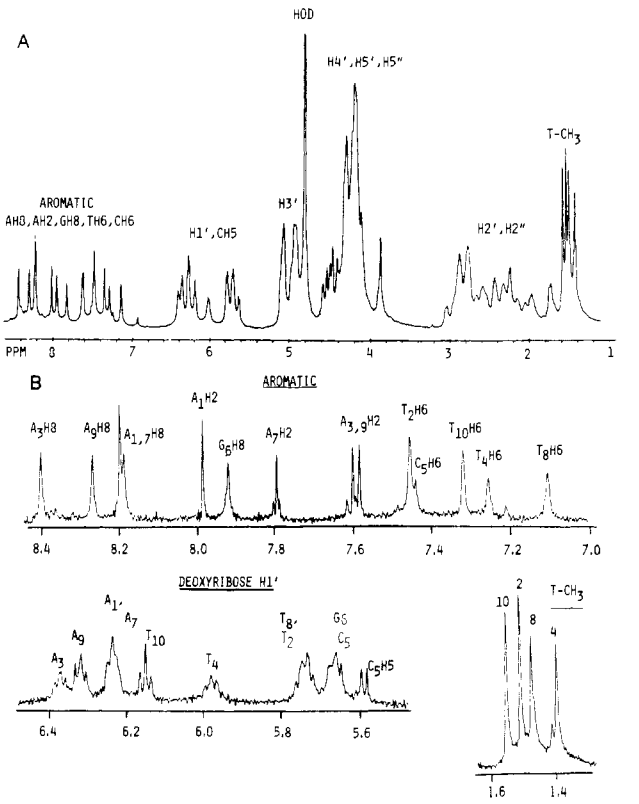


FIGURE 1: (A) 500-MHz ^{1}H NMR spectrum of the nonexchangeable proton resonances in d(ATATCGATAT)$_2$ at 27 °C. A sweep width of ±2000 Hz was used, and the spectrum is line broadened (exponential multiplication) by 2 Hz. Assignments to general proton type are given above the appropriate spectral regions. (B) Resolution-enhanced version of the 500-MHz spectrum given in Figure 1A, showing the aromatic, sugar H1′–CH5, and methyl resonance regions of d(ATATCGATAT)$_2$. The assignments, given above each peak, are discussed in the text.

cross peaks are due to interactions between J-coupled protons. In practice, it is important that the first pulse be 90°, but for various reasons it is desirable to use a "mixing pulse" (second 90° pulse) of around 60° (Bax & Freeman, 1981). Axial peaks were suppressed by a 16-step phase cycling routine; quadrature detection was used in both dimensions, and the carrier was placed at the center of the spectrum as in the 2D-NOE experiments. Details of the data processing are given in the figure caption for the COSY spectrum.

Results

One-Dimensional Spectrum at 500 MHz. A 500-MHz one-dimensional spectrum of d(ATATCGATAT)$_2$ at 27 °C in D_2O is shown in Figure 1A, with general assignments to proton type. Resolution-enhanced spectra of the aromatic, deoxyribose H1′, and methyl regions with specific assignments are given in Figure 1B. The majority of the aromatic resonances were assigned by using one-dimensional NMR techniques described in the preceding paper (Feigon et al., 1983). Completion of these and the methyl assignments, as well as assignments of the ribose H1′ protons, is discussed below.

2D-NOE Spectra. A stacked plot of a 2D-NOE experiment on d(ATATCGATAT)$_2$ at 28 °C is shown in Figure 2. The large diagonal spectrum, which looks similar to the usual one-dimensional spectrum, arises from protons which did not cross relax with other protons during the mixing time. The relatively small peaks appearing off the diagonal are the features of major interest in the 2D spectrum. These cross peaks occur in pairs situated symmetrically off the diagonal and arise from dipole–dipole (or chemical exchange) induced

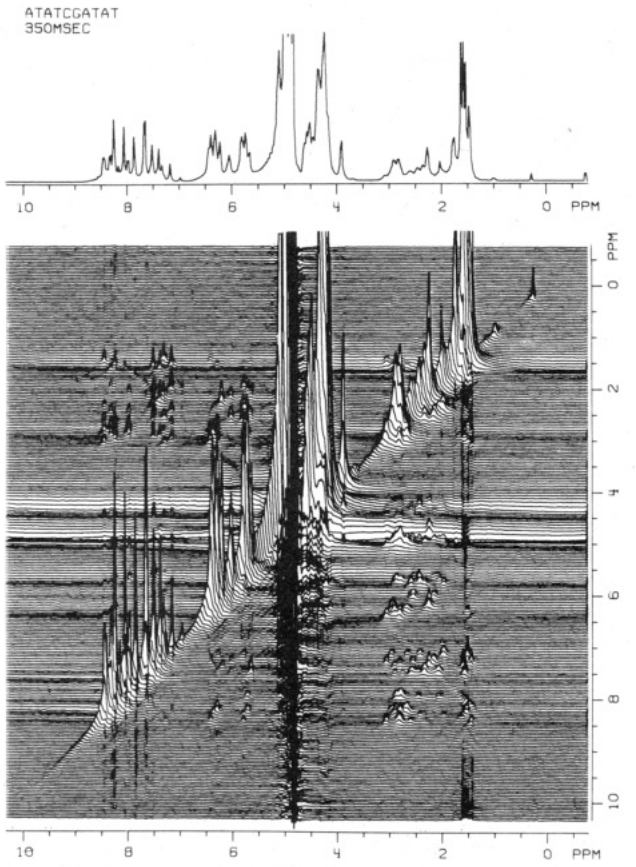


FIGURE 2: Pure absorption mode 360-MHz NOESY spectrum (stacked plot) of d(ATATCGATAT)$_2$ at 28° C in D_2O with τ_m = 350 ms. The spectral width was ±2000 Hz. The data consisted of 1024 points in the t_1 dimension and 128 points in the t_1 dimension; 96 FIDs were accumulated for each value of t_1, with a 4-s delay between acquisitions and 15-h total acquisition time. The resulting data matrix was processed by using a line broadening (exponential multiplication) of 6 Hz in the f_2 dimension and both exponential multiplication and trapezoidal multiplication (with horizontal slope for the first part of the data) as well as one zero filling in the f_1 dimension. Three lines in the stacked plot have been zeroed out in the region of the carrier. A small "ghost" spectrum appears on the diagonal opposite the main spectrum.

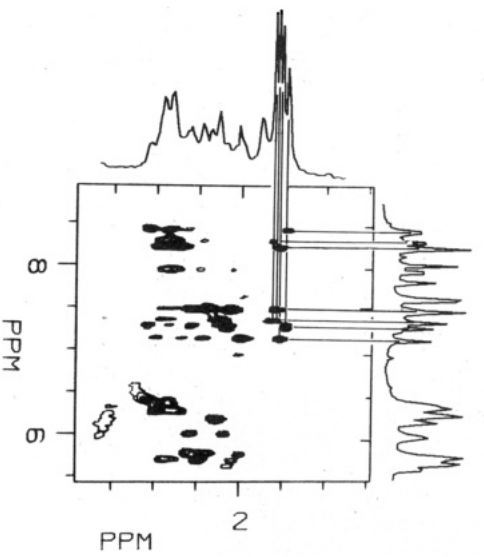


FIGURE 3: Contour plot of the NOESY spectrum given in Figure 2, showing the region of cross peaks between the aromatic and H2′,H2″ sugar photons and the methyl resonances. Cross peaks arising from interactions of AH8 and T-CH_3 protons are specifically indicated.

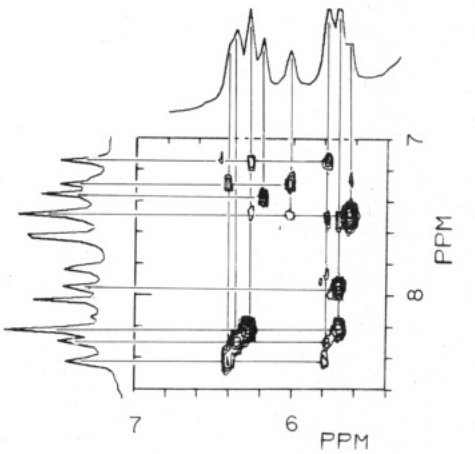


FIGURE 4: Contour plot of the NOESY spectrum given in Figure 2, showing the region of cross peaks between the sugar H1′ and the aromatic resonances. Specific interactions are indicated by the solid lines.

cross relaxation during τ_m (neglecting J-coupled coherences). Thus, in the absence of second-order NOEs, a pair of cross peaks located at (f_1, f_2) and (f_2, f_1) implies a close interaction (probably less than 3.5 Å) between the two protons with (f_1, f_1) and (f_2, f_2) (Kumar et al., 1980a).

In the 2D-NOE spectrum for d(ATATCGATAT)$_2$ numerous cross peaks can be seen, including cross peaks between the aromatic (AH8, GH8, CH6, TH6) and the sugar H2′,H2″ resonances, between the thymine methyl and the TH6 and AH8 resonances, and between the CH5 and CH6 resonances. A contour plot of the region of cross peaks between the aromatic and the sugar H2′,H2″ and the methyl protons is shown in Figure 3. H2′,H2″ cross peaks appear only slightly off of the diagonal, since these resonances occur in the same spectral region, and are, therefore, difficult to distinguish. Some very small cross peaks between the aromatic and the sugar H1′ proton resonances are present, but only at longer mixing times. A contour plot of the H1′ sugar-aromatic region is shown in Figure 4. All of the interactions mentioned above will be considered further under Discussion.

NOE as a Function of Mixing Time. The mixing time (τ_m) for the 2D-NOE spectrum shown in Figure 2 was 350 ms. The initial buildup rates of NOEs in large molecules are directly related to the inverse sixth power of the distance between the irradiated and observed protons (Noggle & Shirmer, 1971; Kalk & Berendsen, 1976; Bothner-By & Noggle, 1979), and this is also true for the 2D NOEs. Therefore, analysis of cross peaks obtained at a single value of τ_m can only give a qualitative picture of proton–proton interactions (Kumar et al., 1981). Furthermore, we note that in general peak heights cannot be used to compare relative intensities, since broad peaks will appear less intense than sharp peaks of the same integrated intensity. Therefore, in the present work we only attempt to make qualitative structure deductions. However, 2D-NOE spectra were also obtained at several different mixing times (60, 100, 150, 350, and 500 ms) (data not shown), and these demonstrate that only a few of the cross peaks that appear in the 350- and 500-ms spectrum can be seen in the 60-ms spectrum (Feigon, 1982; J. Feigon, unpublished results).

2D-J-Correlated Spectra. A 2D-J-correlated (COSY) spectrum of d(ATATCGATAT)$_2$ is given in Figure 5. The spectrum appears similar to the 2D-NOE spectra except that the off-diagonal cross peaks are between homonuclear J-coupled proton resonances (Aue et al., 1976; Nagayama et al., 1979; Jeener et al., 1979). The protons in DNA that are strongly J coupled include the sugar protons (H1′–H2′,

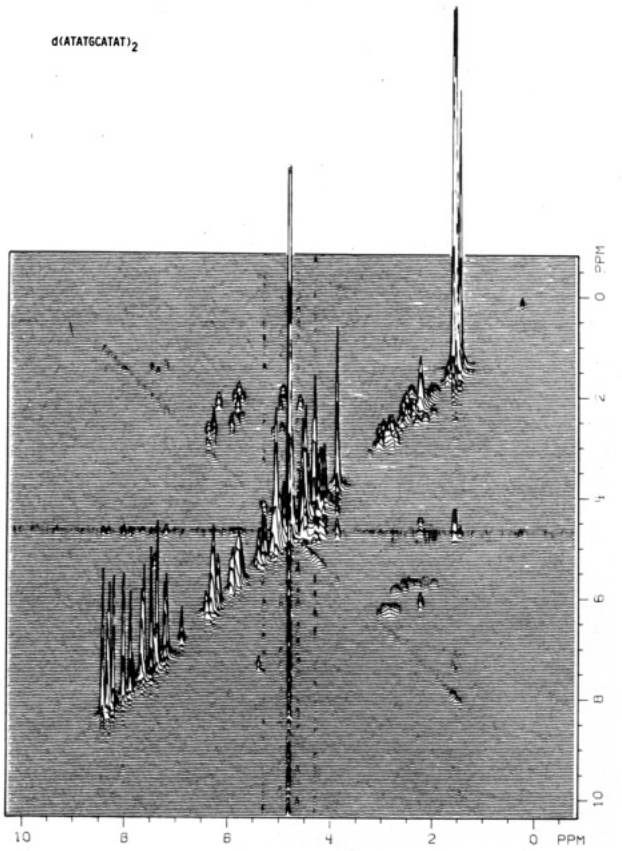


FIGURE 5: 360-MHz COSY spectrum (stacked plot) of d(ATATCGATAT)$_2$ at 29 °C. The spectral width was ±2000 Hz. The data set consisted of 1024 points in the t_2 dimension and 128 points in the t_1 dimension; 96 FIDs were accumulated for each value of t_1, with a 4-s delay between acquisitions. A 90° pulse (13.5 μs) was used for P_1, but P_2 was 9.5 μs. The resulting data matrix was processed with a phase-shifted sine-bell in both dimensions and was zero filled in the f_1 dimension. The absolute value mode is used. An artifactual ghost spectrum appears along the diagonal opposite the main spectrum.

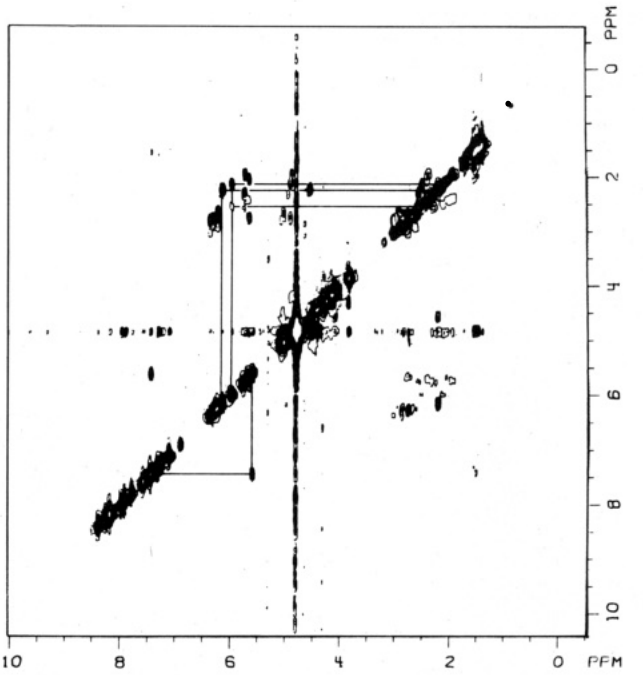


FIGURE 6: Contour plot of the same COSY spectrum of d(ATATCGATAT)$_2$ shown as a stacked plot in Figure 5. The lines connect some of the H1′ and H2′ or H2″ resonances giving rise to cross peaks as well as indicating the CH5–CH6 interaction. The ghost diagonal has been whitewashed.

H1′–H2″, H2′–H3′, H2″–H3′, H3′–H4′, H4′–H5′, H4′–H5″) and CH5–CH6. All of these interactions are clearly seen in the spectrum, although for the coupled sugar protons only the H1′–H2′,H2″ cross peaks are resolved. In addition, very small cross peaks can be seen between the T-CH3 resonances and the TH6 resonances due to the long-range coupling between these resonances. This sets an approximate lower limit on couplings which will give rise to observable cross peaks under these experimental conditions, since the T-CH$_3$–TH6 coupling constants have been measured in pT to be ~1.2 Hz (Wood et al., 1974). A contour plot of the spectrum shown in Figure 5 is given in Figure 6.

Discussion

In this paper we have used the COSY and NOESY 2D-NMR techniques to study the double-stranded DNA decamer, d(ATATCGATAT)$_2$. The COSY experiments were used to confirm spectral assignments deduced from 1D-NMR studies and obtain new assignments which could be difficult to obtain by using just 1D techniques. The NOESY experiments also provide new information on assignments (via spatial proximities), but more importantly, they provide a qualitative picture of the spatial proximities of various protons in the molecule, and hence structural information. The dynamic properties of the DNA are also evident in certain aspects of the 2D-NOE spectra.

Analysis of the COSY Spectrum. A large cross peak is observed between a proton resonance in the aromatic region

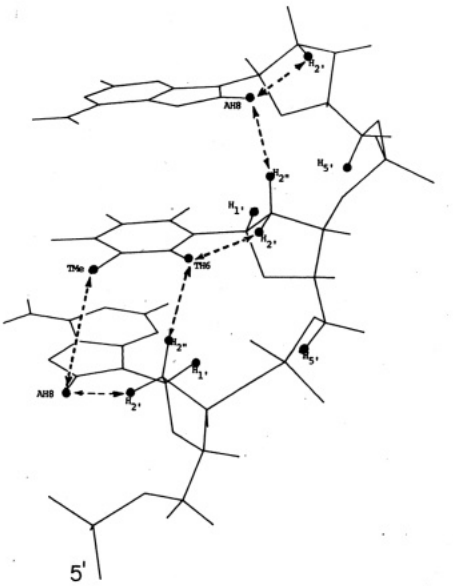
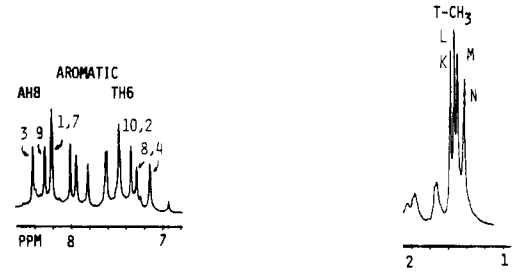


FIGURE 7: Diagrammatic representation of some of the interproton interactions which could give rise to NOEs in B-DNA in general and d(ATATCGATAT)$_2$ in particular. Note that the thymine methyl protons interact with base protons of the 5′-neighboring nucleotide, but not with base protons of the 3′-neighboring nucleotide. Thus, the methyl on T_2 interacts with H8 on A_1, but not with H8 on A_3. Note also that the purine H8 and pyrimidine H6 protons can interact both with the H2′ sugar proton of the same nucleotide and the H2″ proton of the 5′→3′ neighbor nucleotide unit.

and one in the 5–6 ppm region (Figure 7). These resonances had previously been assigned to C_5H6 and C_5H5, respectively (see preceding paper), and the results obtained here reconfirm that assignment. Each H1′ proton exhibits two cross peaks corresponding to the interactions with H2′ and H2″ protons of the same residue. Cross peaks due to H2′–H2″ interactions appear only slightly off the diagonal since both these resonances appear in the same spectral region. The H2′,H2″ protons also show cross peaks with the H3′ protons. Because

Table I: Assignment of the AH8, TH6, and T-CH_3 Resonances

$A_1\ T_2A_3T_4C_5G_6A_7T_8A_9\ T_{10}$
$T_{10}\ A_9T_8A_7G_6C_5T_4A_3T_2A_1$



assignments based on 1D NMR	proton	ppm	cross peak with methyl: assignment	ppm	base	final assignments based on 2D NMR
3 or 9	AH8	8.39	N	1.39	$T_4^{\wedge}$ or T_{10}	3
3 or 9	AH8	8.26	K	1.55[a]	T_4 or T_{10}	9
1, 7	AH8, AH8	8.18	(L), M	1.51 (1.47)[a]	T_2, T_8	1, 7
10 or 2	TH6	7.45	L	1.51	T_2 or T_8	2
	CH6	7.45				
10 or 2	TH6	7.32	K	1.55	T_{10}	10
8 or 4	TH6	7.25	N	1.39	T_4	4
8 or 4	TH6	7.10	M	1.47	T_2 or T_8	8

[a] Small cross peak.

all protons within any given sugar are sequentially coupled, it would be possible, given adequate resolution, to identify all of the sugar proton resonances associated with a particular (albeit unassigned) sugar. Therefore, once any sugar proton in a given nucleotide is assigned, all of the rest can be assigned by using the COSY spectrum. However, with the resolution of the experiments shown here, only the H1′–H2′,H2″ connectivities can be made for the majority of the sugars, and some of these are shown in Figure 6.

Analysis of the NOESY Spectra: Comparison of NOESY and COSY Spectra. Cross peaks which appear in the COSY spectrum due to *J* coupling can also appear in the NOESY spectrum as a result of dipole–dipole interaction between these closely spaced protons. These peaks can be distinguished from other cross peaks in the NOESY spectrum that arise from through-space dipole–dipole interactions between nonbonded (i.e., via carbon atoms) protons by comparing the COSY and NOESY spectra.

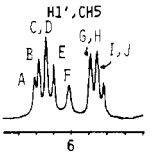
Cross peaks in the NOESY spectrum arising from interactions between ribose protons give essentially the same connectivities as the *J*-coupled cross peaks obtained in the COSY spectrum. In addition, the H5′,H5″ sugar protons are expected to exhibit NOEs with the neighboring sugar proton resonances (5′→3′ strand direction), most likely by interaction with the H2″ on the neighboring sugar. Since the terminal TH1′ resonance can be assigned (see below), the information contained in the NOESY and COSY spectra can, in principle, be used to assign all of the sugars along the strand. Unfortunately, better resolved spectra will have to be obtained before this is actually possible in practice. The H3′, H4′, H5′, and H5″ resonances all occur in an overlapping spectral region, making identification of cross speaks virtually impossible. Further discussion of the sugar cross speaks is, therefore, limited to those involving the H1′ and H2′,H2″ resonances.

Assignments of T-CH_3 and TH6 and AH8 Resonances. Some of the strongest cross peaks in the 360-MHz spectrum shown in Figure 4 are between the thymine methyl resonances and the aromatic TH6 and AH8 resonances. The strong cross peaks connecting the four methyl resonances and the four TH6 resonances result from *intra*base interactions between the TH6 and the rotating methyl protons which are about 2.5 Å away (at closest approach). The same pattern of interactions between the T-CH_3 and the TH6 resonances was observed in the COSY spectrum, due to long-range coupling between the methyl protons and the TH6. In addition to the intrabase cross peaks, there are three or four (two of the AH8 resonances overlap) *inter*base cross peaks between the AH8 and the T-CH_3 protons. The existence of these cross peaks indicates that the AH8 protons are near in space to the thymine methyl protons, and the fact that the intensities for at least two of the T-CH_3–AH8 cross peaks are comparable to the intensities of the T-CH_3–TH6 cross peaks implies that the interproton distances are similar.

Examination of a model of B-DNA indicates that for an alternating A·T sequence, the protons on the methyl group on a T base could approach within 2.6 Å from the H8 proton on the A base located on the 5′ side (i.e., ApT) but are much further away (>5 Å) from the A base on the 3′ side (i.e., TpA). This is illustrated schematically in Figure 7. For d-$(ATATCGATAT)_2$, cross peaks would be expected between the T-CH_3 and AH8 resonances of T_2 and A_1, T_4 and A_3, T_{10} and A_9, and T_8 and A_7. It has already been noted that there are cross peaks between the TH6 and T-CH_3 of the same residue, and therefore, if the assignments of either the T methyl resonances, the TH6 resonances, or the AH8 resonances are known, all of the others can be deduced from the 2D spectrum.

By use of this information, and the partial assignment of the aromatic proton resonances made in the previous paper, all of the aromatic and methyl resonances were assigned, and the results are shown in Table I. As an example, we note that the assignment of the AH8 resonance at 8.26 ppm was either to base 3 or 9 on the basis of the 1D NMR data; however, on the basis of the 2D data, this resonance is clearly due to residue A_9 as the following arguments demonstrate. If the 8.26 ppm resonance is due either to A_3 or A_9, then the cross peak to methyl resonance K must therefore be from base 4 or 10. The TH6 resonance at 7.32 ppm, which had been assigned to base 10 or 2, also shows a cross peak to methyl K. By process of elimination, the TH6 resonance at 7.32 and methyl resonance K are assigned to base A_9. Similar arguments lead to the other assignments displayed in the right-handed column of Table I.

Table II: Assignment of H1′ Resonances



sugar resonance	ppm[b]	cross peaks	assignment
A	6.36	A_3H8; T_4H8	A_3
B	6.32	A_9H8	A_9
C, D[a]	6.23	A_1H8 or A_7H8; T_2H6 or C_5H6; T_8H6	A_1; A_7
E	6.15	T_{10}H6	T_{10}
F	5.98	T_4H6; T_2H6 or C_5H6	T_4
G, H[a]	5.74	A_3H8; A_9H8; T_2H6 or C_5H6; T_8H6	T_8; T_2
I, J[a]	5.67	A_1H8 or A_7H8; G_6H8; T_2H6 or C_5H6	G_6; C_5

[a] Where two peaks overlap, both are listed. [b] $T = 30$ °C.

It is of interest to note that although there are four strong methyl–TH6 interactions, only two of the four (or three) methyl–AH8 interactions are relatively strong. Examination of the assignments reveals that the weaker interactions are between the base pairs at the ends of the helix. This is attributed to transient opening of the base pairs at the ends of the helix and is discussed further below.

Assignments of the Sugar Resonances. Once the aromatic resonances have been assigned, all of the sugar resonances can (in principle) be assigned on the basis of NOEs between the aromatic and sugar protons. The method discussed below for assigning H1′ sugar resonances using the results of the 2D-NOE experiments is of considerable interest, since there have been no reliable assignments of sugar protons in synthetic double-stranded DNA (Patel & Canuel, 1977; Patel & Hilbers, 1975) until recently (Cheng et al., 1982; Kan et al., 1982). All of the aromatic protons, except the AH2, show small cross peaks with the deoxyribose H1′ protons at long mixing times (350 ms), and in many cases there is more than one interaction. For comparison, the largest cross peak in this region is due to the CH5–CH6 interaction (~2.4 Å). If the sugar conformations are S type and the bases anti, as indicated by the experimental results (see below), then the H1′ proton in a particular nucleotide will be ~3.5 Å away from the purine H8 or pyrimidine H6 proton on its attached base. The H2′ proton will be considerably closer to the base (H8 or H6) proton, and it is probable that the observed cross peaks between the H1′ and base proton (H8 or H6) resonances arise from second-order NOEs involving the H2′,H2″ protons. In any case, whether the H1′-base (H8 or H6) cross peaks are due to first- or second-order NOEs, in B-form DNA these (H1′ as well as H2′,H2″) sugar protons could interact both with the protons of their own base and with their 3′ neighbor base, but *not* with the 5′ neighbor base as illustrated in Figure 7.

Many of the H1′ resonances do show cross peaks with more than one base, and this makes it possible to assign most of the H1′ resonances to particular bases since the aromatic resonances can be assigned (Table II). As an example of how the assignments were made, consider the sugar H1′ resonance A at 6.36 ppm in Table II. Cross peaks between the H1′ proton resonance (A) and A_3H8 as well as T_4H6 were observed. This makes it possible to immediately assign this H1′ to base A_3 (provided the DNA helix is right-handed and in the A, B, C structure range), since the sugar protons on A_3 could interact with their own base H8 proton and with the aromatic proton on T_4, but the sugar protons on T_4 could only interact with their own aromatic proton and/or the aromatic proton of C_5. Because some of the aromatic resonances overlap, in particular, A_1H8 and A_7H8 as well as T_2H6 and C_5H6, some of the assignments are uncertain. However, a higher resolution spectrum should resolve some of these uncertainties. One resonance that could be assigned unambiguously was that for the terminal T. This resonance appears sharper than all the other H1′ resonances and appeared to be slower relaxing in a T_2 experiment [see Feigon et al. (1983)]. Since the terminal T is at the 3′ end, the H1′ proton can only interact with its own base, and only one small cross peak was seen. Furthermore, only one relatively intense cross peak is observed between this H1′ resonance and the H2′,H2″ resonances in both the COSY and NOESY spectra. This is consistent with the fact that the H2′ and H2″ resonances in pT both appear at the same frequency (Wood et al., 1974).

Assignment of the terminal AH1′ should also be straightforward, since the H8 on A_1 could only interact with its own sugar protons. Unfortunately, the A_1H8 and A_7H8 resonances overlap in d(ATATCGATAT)$_2$, with the result that two sets of cross peaks are observed. One of these cross peaks, that between $A_{1,7}$H8 and the overlapping H1′ C,D resonances, is more intense than any of the others, so it was of some interest to confirm that this was at least partially due to the A_1H8 interaction with H1′. To accomplish this, the results obtained on d(ATATCGATAT)$_2$ were compared with those for d(ATATGCATAT)$_2$ (not shown), since A_1H8 and A_7H8 are resolved in the latter decamer. The A_1H8 resonance shows only one cross peak with the H1′ region, and this is to a resonance at the same chemical shift as those labeled C, D in the "CG" decamer. If we make the plausible assumption that the conformations of the terminal A sugars are the same in both decamers, then this unambiguously confirms the assignment of the H1′ resonances at 6.23 ppm to nucleotide A_1. The relatively large intensity (and appearance at short τ_m) of the A_1H8–H1′ cross peak was somewhat unexpected, especially when compared to the intensity of the A_1H8–H2′,H2″ cross peaks. This may indicate a somewhat different conformation for the terminal A sugar compared to the other sugars, but this is not too surprising in view of the additional flexibility available to the terminal sugar.

Obviously, once the H1′ resonances are assigned, the rest of the sugar protons can in principle be assigned by using the connectivities manifested in the COSY (or NOESY) spectrum.

Interaction between the H2′,H2″ and Aromatic and H1′ Protons. Four cross peaks are observed in the NOESY spectrum for each aromatic resonance which interacts with the H2′,H2″ resonances. Two of these cross peaks are intense, while the other two are generally only slightly more intense than the H1′-aromatic cross peaks. As illustrated in Figure 7 and discussed further below, the aromatic (H6 and H8) proton on a given base is near in space both to its own sugar H2′ protons and to the H2″ proton of the neighboring 3′ nucleotide. The terminal AH8 would of course only be near its own sugar protons since it lacks a 3′ neighbor. When the cross peaks seen between the aromatic and the H2′,H2″ resonances are carefully compared with the H1′–H2′,2″ cross peaks, it can be seen that the two stronger interactions always correspond to one of the pairs of interactions observed between each H1′ and H2′,H2″ resonances in the NOESY and COSY spectra. This means that the more intense cross peaks arise from *intra*nucleotide interactions of each aromatic proton with the H2′ and H2″ protons in one sugar residue, while the less intense cross peaks are from interactions with the H2″ protons

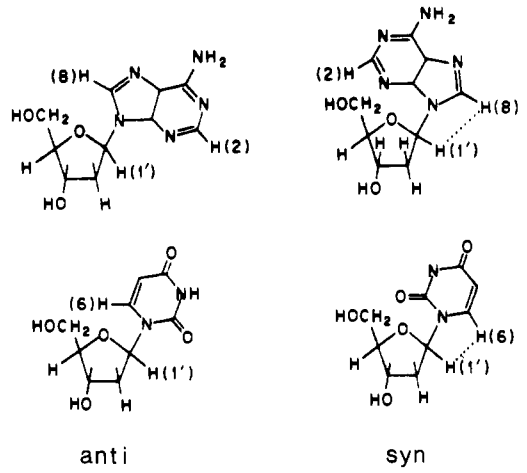


FIGURE 8: Diagrammatic representation of glycosidic bond syn and anti conformations for both purine and pyrimidine nucleotide units illustrating the close approach of the H1′ protons to base protons in the syn conformation.

of the sugar of the nucleotide on the 5′ side.

If the strongest interactions are between the base and its own sugar, then the assignments for the H1′ resonances shown in Table II are completely consistent with the assignments that would be made by comparing the deoxyribose H1′–H2′,H2″ cross peaks with the base–H2′,H2″ cross peaks.

Further evidence in support of these assignments is found in the recent work of Tran-Dinh et al. (1982). They assigned the sugar proton resonances in the single-stranded DNA fragments d(ApTpGpT) and d(ApCpApTpGpT). Purine H2′,H2″ resonances are found to resonate from ~2.6 to 2.8 ppm, while pyrimidines resonate from ~1.9 to 2.4 ppm. Although the H2′,H2″ resonances shift downfield during the helix-to-coil transition, examination of the chemical shifts of the H2′,H2″ resonances in d(ATATCGATAT)$_2$ as a function of temperature reveals that the higher field group of resonances remains upfield from the lower field group of resonances throughout the duplex-to-strand transition. This indicates that the purine H2′,H2″ resonances will, in general, be found downfield of the pyrimidine resonances. Furthermore, it was noted by Tran-Dinh et al. (1982) that the H2′,H2″ resonances of the internal purine residues have very similar chemical shifts, while the H2′,H2″ resonances of the internal pyrimidine residues are well separated. Obviously care must be taken in extrapolating those observations in single-stranded DNA to double-stranded DNA. Nevertheless, these two observations are completely consistent with the results obtained on d(ATATCGATAT)$_2$, as can be seen by observation of the cross peaks between the H2′,H2″ resonances and the H1′ resonances.

Structural Features of d(ATATCGATAT)$_2$: Evidence for a B-Type Conformation. Among the most dominant cross peaks in the NOESY spectra, especially at the shorter mixing times, are the cross peaks between the H2′,H2″ proton resonances and the aromatic AH8, GH8, TH6, and CH6 resonances (also the H3′ resonances). In contrast, cross peaks between the H1′ resonances and the aromatic region are reelatively very small (see Figure 2) and can only be seen clearly at long mixing times. The single exception is the cross peak between A_1H8 and the terminal H1′. The weakness of the H1′-aromatic cross peaks is significant in light of the extensive conformational studies on nucleosides and nucleotides using NOEs [for reviews, see Davies (1978) and Bothner-By (1979)]. For the anti conformation (Figure 8), a strong NOE is expected from the H2′ to the purine H8 or pyrimidine H6, while for the syn conformation a strong NOE is expected from the H1′ to the purine H8 or pyrimidine H6. The situation is more complicated in DNA due to the possibility of additional NOEs between the sugar protons of one nucleotide and the protons on a neighboring nucleotide. For example, examination of a model of B-DNA indicates that, depending on the exact sugar pucker and base–sugar torsion angles, the aromatic protons (AH8, GH8, CH6, and TH6) could interact both with their own sugar H2′,H2″ protons and with the H2′,H2″ protons from the neighboring sugar (5′→3′ strand) (Figure 7). However, a strong NOE between the base and H1′ protons would only be expected for the syn conformation. While care must be taken when peak intensities in 2D-NOE spectra are compared, the much larger intensities of the H2′,H2″-aromatic cross peaks compared to the H1′-aromatic cross peaks, as well as the fact that H2′,H2″-aromatic cross peaks appear as early as τ_m = 60 ms (data not shown), make it reasonable to conclude that with the possible exception of the terminal A_1H8 nucleotide all the purine and pyrimidine bases in this decamer are in the anti conformation.

The coupling constants measured for the H1′ sugar resonances in d(ATATCGATAT)$_2$ provide additional evidence for the B-DNA conformation for this decamer [see preceding paper (Feigon et al., 1983)]. These values were consistent with a predominantly S-type sugar conformation (C2′-endo, C3′-exo) (Altona & Sundaralingam, 1973; Haasnoot et al., 1981) for the sugars in the molecule, and this is the conformation expected for B-family DNAs (but not for A-DNA or the pyrimidines in Z-DNA). The experimental evidence for the anti conformation for the bases combined with an S-type sugar pucker places important restrictions on the conformation of the decamer and makes its classification into the B-family quite reasonable.

Other Interactions with Aromatic Protons. The relative intensities of cross peaks in the 2D-NOE spectrum can be used to qualitatively estimate interproton distances. For example, the CH5–CH6 distance is fixed at 2.4 Å, so other cross peaks with similar intensities are expected from protons separated by a comparable distance. Note, however, that NOEs to either one of the H2′ or H2″ protons in a given sugar will generally result in comparably intense cross peaks for both resonances, due to rapid cross relaxation between the strongly coupled H2′ and H2″ protons.

No cross peaks are observed between the AH2 resonances and any sugar resonances in the molecule. Since the AH2 protons are at least 5 Å away from the H1′ protons (and even further from any other sugar protons), this sets an upper limit to the interproton distances which result in observable cross peaks in these molecules, even in the presence of spin diffusion. As discussed in the preceding paper (Feigon et al., 1983), relaxation data indicate there is a moderately strong cross-strand interaction between A_3H_2–A_9H protons. An analogous interaction is observed in poly(dA-dT) (R. Behling and N. Assa-Munt, unpublished results). This arises from the interaction between AH2 protons of adenine bases in adjacent base pairs but situated in opposite strands of the helix in a TpA sequence, but not the ApT sequence (Dickerson & Drew, 1981; S. Arnott, private communication). Unfortunately the AH2–AH2 interaction cannot be observed in the NOESY spectrum of d(ATATCGATAT)$_2$ because the resonances for these two protons overlap.

Cross peaks between the purine H8 and the H1′ resonances were generally observed to be larger than those between the pyrimidine H6 and the H1′ resonances. This is likely due in large part to the fact that the pyrimidine H6 protons have large

dipolar interactions with their own methyl group or proton at the 5-position in T and C, respectively. However, another contributing factor could be due to differences in conformations between the two types of nucleotides. Dickerson & Drew (1981) have pointed out that the CH6 and CO2 atoms place steric restrictions on the conformation that the sugar can assume relative to the base. This means that purine sugars have a conformation closer to the classical C2′-endo B-DNA helix than do pyrimidines. If this is so, then the H2′ is likely to be closer to the H8 on purines than the H6 on pyrimidines (intranucleoside interaction).

Dynamics of the Double Helix: Opening of the Base Pairs near the Ends of the Helix. In a preceding section it was noted that of the four cross peaks between T methyl protons and the 5′-neighboring AH8 resonance, the two relatively intense peaks are from A_3–T_4 and T_8–A_7 interactions while the less intense cross peaks are from A_1–T_2 and A_9–T_{10} interactions. Similar results were observed with the d(ATATGCATAT)$_2$ decamer, where the A_1H8 and A_9H8 resonances, which overlap in the d(ATATCGATAT)$_2$ decamer, are resolved. The obvious implication is that the average distance between the T methyl and the AH8 on the terminal and next-to-terminal base pairs is greater than that for corresponding protons in the interior base pairs.

In the low-field spectra of these molecules at 28 °C, the imino proton resonances from the terminal and next-to-terminal base pairs of the decamer are broadened to base line, indicating rapid exchange of these resonances with water on the NMR time scale. By contrast, the more interior A·T and central G·C imino resonances remain sharp [Figure 2B (Feigon et al., 1983)].

The broadening of the imino resonances gives information on the kinetics of base pair opening but tells nothing about the equilibrium position of the base pairs. The results of the 2D-NOE experiment give information on the time averaged conformation of the decamer. Because NOEs are inversely related to the sixth power of the internuclear distance, most of the NOE observed will be due to interactions between the protons at their distance of closest approach. Furthermore, it has already been noted that cross peaks in these molecules will only be observed between protons which are <5 Å apart. Therefore, the fact that cross peaks, albeit small ones, are observed at all between the methyl resonances on T_2 and T_{10} and the H8 resonances on A_1 and A_9, respectively, indicates that terminal and next-to-terminal base pairs remain primarily stacked at 28 °C. The temperature dependence of the aromatic proton chemical shifts indicates that the molecule remains primarily double stranded until at least 40 °C [see preceding paper (Feigon et al., 1983)]. A detailed 2D-NOE study of this decamer as a function of temperature should prove quite useful in following the equilibrium conformational changes that occur during premelting and melting of the double helix.

Conclusions

In this paper, 2D-NOE and 2D-*J*-correlated spectra were used to obtain information on the conformation of a double-stranded DNA and to make assignments of the resonances, which would have been difficult or impossible to obtain by using other NMR methods. The NOE cross peaks observed were consistent with the anti conformation for the bases, which is expected for B-form DNA. Furthermore, in both d(ATATCGATAT)$_2$ and d(ATATGCATAT)$_2$, the observation of a cross peak between the terminal AH8 and the methyl on a (neighboring T) base indicates that the methyl group must be near in space to the terminal AH8 and thereby implies that the base pairs are in the correct spatial configuration for a right-handed B-DNA helix. This information, combined with coupling constants for the H1′ protons ($J_{1'2'} + J_{1'2''}$) which indicate a predominantly S-type sugar pucker for the molecule, leaves little doubt that the d(ATATCGATAT)$_2$ has a B-family conformation. The NOESY experiment also provided information that made it possible to complete the assignments of the base proton resonances. A new method for assigning the H1′ sugar resonances, based on the small cross peaks observed between the base H8 and H6 resonances and the H1′ resonances, was presented. Once the H1′ resonances are assigned, the results of a COSY experiment can be used to assign the other sugar proton resonances. The cross peaks observed between the H2′,H2″ resonances and the base H8 and H6 resonances were consistent with those expected based on the H1′ assignments. If the assignments made here are correct, then the cross peaks between H2′,H2″ and the base protons were always more intense for the intranucleotide vs. the internucleotide interaction, and this implies that the H2′ proton in a given sugar is nearer to its own base than the H2″ proton on that same sugar is to the 3′-neighboring base.

Now that important interproton interactions have been identified by NOE, it should be possible to probe structural changes in the DNA due to bound ligands. Such experiments using site-specific drugs with slow exchange kinetics are in progress. It is hoped that this type of study will make it possible to determine the precise orientation of a drug in a binding site as well as changes in the DNA conformation which occur in order to accommodate the bound drug.

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Optical Properties and Small-Angle Neutron Scattering of Bovine Heart Mitochondrial Oligomycin Sensitivity Conferring Protein†

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ABSTRACT: The mitochondrial oligomycin sensitivity conferring protein (OSCP) was isolated from beef heart mitochondria according to Senior (1979) [Senior, A. E. (1979) *Methods Enzymol. 55*, 391–397] with a supplementary step of Sephadex chromatography for more extensive purification. The ultraviolet and fluorescence spectra of OSCP were consistent with the presence of tyrosyl residues and the absence of tryptophanyl residues. From the circular dichroism spectrum of OSCP, 43% α-helical structure was calculated; the dichroism spectra of OSCP in H_2O and D_2O were identical. A molecular weight (M_r) of 22 000 for OSCP was determined by sodium dodecyl sulfate gel electrophoresis at different concentrations of the polyacrylamide gel. The radius of gyration (R_g) and the shape of OSCP in H_2O and D_2O were studied by small-angle neutron scattering. The experimentally determined R_g value of OSCP in H_2O was 24 ± 1 Å, and its M_r was 25 000 ± 3000. Comparison of the experimental R_g value with that expected for a compact globular protein of the same molecular weight (17 Å) led to the conclusion that OSCP is a considerably elongated molecule protein with an axial ratio higher than 3. In D_2O buffer, the R_g value was higher than that in H_2O, a situation in contrast with that observed for most globular hydrophilic proteins; this might be due to a preferential location of the positively charged lysine residues.

The oligomycin sensitivity conferring protein (OSCP),[1] a water-soluble, basic protein, is one of the subunits of the mitochondrial F_1–F_0 ATPase complex. As shown in reconstitution experiments, OSCP confers oligomycin sensitivity to F_1 and is essential for restoration of energy-linked functions such as the ATP–P_i exchange reaction or the ATP-driven reversal of electron transport. It has been proposed that OSCP not only has a structural role as a connecting link between the F_1 and F_0 sectors of the ATPase complex but also has a functional role, either directly conducting protons or mediating conformational changes between the two sectors of the complex (Tzagoloff et al., 1968; Mac Lennan & Tzagoloff, 1968; Tzagoloff, 1970; Senior, 1971, 1979). OSCP is absent in

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[1] Abbreviations: OSCP, oligomycin sensitivity conferring protein; F_1, soluble coupling factor 1; F_0, membrane sector of the ATPase complex; $NaDodSO_4$, sodium dodecyl sulfate; ASUA, submitochondrial particles isolated in the presence of ammonia and successively treated with Sephadex, urea, and ammonia; Tris, tris(hydroxymethyl)aminomethane.

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