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Articles

Nuclear Magnetic Resonance Studies of Complex Formation between the Oligonucleotide d(TATC) Covalently Linked to an Acridine Derivative and Its Complementary Sequence d(GATA)

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ABSTRACT: The oligodeoxynucleotide d(TATC) was covalently attached to the 9-amino group of 2-methoxy-6-chloro-9-aminoacridine (Acr) through its 3′-phosphate via a pentamethylene linker (m_5). Complex formation between d(TATC)m_5Acr and the complementary strand d(GATA) in aqueous solution was investigated by nuclear magnetic resonance. The COSY and NOESY connectivities allowed us to assign all the proton resonances of the bases, the sugars (except the overlapping 5′/5″ resonances), the acridine, and the pentamethylene chain. Structural informations derived from relative intensities of COSY and NOESY maps revealed that the duplex d(TATC)–d(GATA) adopts a B-type conformation and that the deoxyriboses preferentially adopt a 2′-endo conformation. The NOE connectivities observed between the protons of the bases or of the sugars and the protons of the dye and of the pentamethylene chain led us to propose a model involving an equilibrium between two families of configurations. In the first family, the acridine derivative is intercalated between base pairs C_4-G_4 and T_3-A_3. In the second family, the acridine derivative is sandwiched between two aggregated duplexes. The structure of the intercalated complex as well as that of the aggregated species is discussed.

Gene expression is usually controlled by specific proteins that recognize a base sequence or a nucleic acid local structure. The interactions between functional groups in protein–nucleic acid complexes have been recently reviewed (Hélène & Lancelot, 1982). The binding of an oligonucleotide to its complementary sequence is a highly specific process governed by

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stacking interactions between base pairs and by hydrogen-bond formation between complementary bases. In order to achieve higher affinity without losing the specificity of base-pair formation, oligonucleotides can be modified in such a way as to introduce additional interactions with the target sequence. We recently described the synthesis of oligodeoxynucleotides covalently linked to 2-methoxy-6-chloro-9-aminoacridine (Acr). A polymethylene linker was used to bridge the 3′-phosphate group of the oligonucleotide to the 9-amino group of the acridine derivative (Asseline et al., 1983, 1984a,b). Absorption studies showed that the presence of the intercalating agent strongly stabilized the complexes formed by the oligonucleotides with their complementary sequences (Asseline et al., 1984). In order to obtain further information on the structure of the complexes formed by an oligonucleotide bearing an acridine derivative and on the interactions engaged by the acridine ring with nucleic acid bases, we undertook a nuclear magnetic resonance investigation of the interaction of d(TATC)m_5Acr[1] with its complementary sequence d(GATA) in aqueous solutions at pH 7. These results show that the acridine ring undergoes stacking interactions with the base pairs in the duplex structure formed by d(TATC) and its complementary sequence d(GATA).

Experimental Procedures

The syntheses of the oligonucleotides used in this study have been described elsewhere (Thuong et al., 1981; Asseline et al., 1983). Covalent attachment of 2-methoxy-6-chloro-9-aminoacridine to the oligodeoxynucleotide involved a chain of five methylene groups linking the 3′-phosphate of the tetranucleotide to the 9-amino group of acridine. This compound will be abbreviated as d(TATC)m_5Acr. Bases will be numbered from the 5′ end. In the complementary oligonucleotide d(GATA), bases will be numbered from the 3′ end.

All the investigated compounds were passed through a Chelex-100 column to remove paramagnetic impurities, adjusted to pH 7.0, then lyophilized, dissolved in D_2O containing 0.2 M NaCl, lyophilized, and dissolved in 99.96% D_2O. The samples were placed in a 5-mm NMR tube, and the spectra were calibrated with respect to an internal reference of 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) for proton spectra and trimethyl phosphate (TMP) for phosphorus spectra. The NOESY data were obtained on degassed solutions contained in sealed tubes.

NMR experiments were carried out with a Bruker AM-300 spectrometer and processed on an Aspect 3000 computer. Two-dimensional data sets for the COSY and NOESY spectra were collected in the phase-sensitive mode by the method of States et al. (1982). The experimental procedures used for COSY DQF experiments were described elsewhere (Marion & Lancelot, 1984).

Typically, 4096 complex t_2 data points were collected for each of 256 t_1 values in NOESY experiments. A mixing time of 300 ms was used. A 512 × 4096 data matrix, sampled in a 48-h experiment, was resolution-enhanced by a Gaussian window function in direction 2 and by a shifted squared "sine-bell" window function in direction 1, then Fourier transformed, and phase adjusted.

Results

The 300-MHz proton spectra of the mixture d(TATC)m_5Acr + d(GATA) in 0.2 M NaCl is shown in Figure

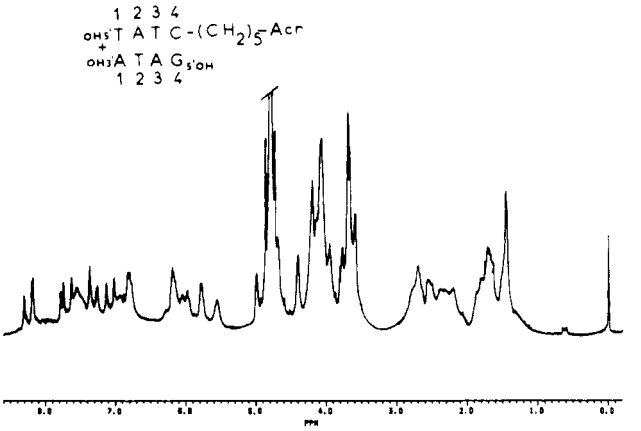

FIGURE 1: The 300-MHz ^1H NMR spectrum of d(TATC)m_5Acr + d(GATA) (7 mM:7 mM) in 0.2 M NaCl, pH 7.0 at 21 °C.

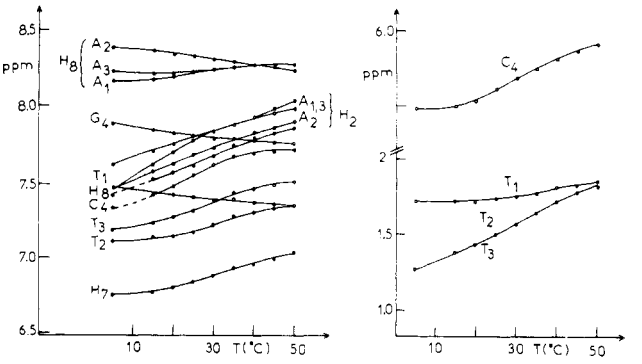

FIGURE 2: Temperature dependence of chemical shifts of the mixture d(TATC)m_5Acr + d(GATA) (3 mM:3 mM) in 0.2 M NaCl, pH 7.0.

1. The mixing of the two compounds induced a broadening of all the resonance lines and several upfield shifts of the aromatic resonances. From the temperature dependence of these proton chemical shifts, a melting temperature of 27 °C was calculated at a strand concentration of 3 mM (Figure 2). Under the same experimental conditions, the mixing of d(TATC) with d(GATA) did not induce any important shift of the aromatic resonances as compared to the free oligonucleotides in the range of temperature 10–60 °C, indicating that only a small amount of duplex structure was formed. Therefore, we conclude that the presence of the acridine dye covalently linked to the oligonucleotide by an aliphatic chain strongly increases complex formation between the two complementary oligodeoxynucleotides. Such a property was already demonstrated for the binding of oligothymidylates to poly(rA) (Asseline et al., 1984a,b) or oligo(rA) (Lancelot et al., 1985). These studies revealed that the stabilization depended on the linker length and increased when the number of methylene groups increased from three to five, with no additional stabilization from five to six (Asseline et al., 1984a). By using two-dimensional NMR methods (NOESY and COSY data), we obtained the complete assignment of all the resonances of the nonexchangeable protons of the bases and of the sugar except for the overlapping 5′ and 5″ resonances. A general analysis of the conformation of the miniduplex is developed below, and the intercalation of the acridine between base pairs is clearly demonstrated.

Assignment of Resonances. The use of the sequential NOE method for the assignment of nonexchangeable resonances in nucleic acids has been described in detail elsewhere (Hare et al., 1983; Scheek et al., 1983, 1984; Feigon et al., 1983, 1984; Weiss et al., 1984; Wemmer et al., 1984, 1985; Guittet et al., 1984; Broido et al., 1984) and will not be repeated here.

[1] Abbreviation: d(TATC)m_5Acr, deoxyribooligonucleotide d(TATC) covalently linked on its 3′-terminal phosphate to 2-methoxy-6-chloro-9-aminoacridine (Acr) via a pentamethylene chain (m_5).

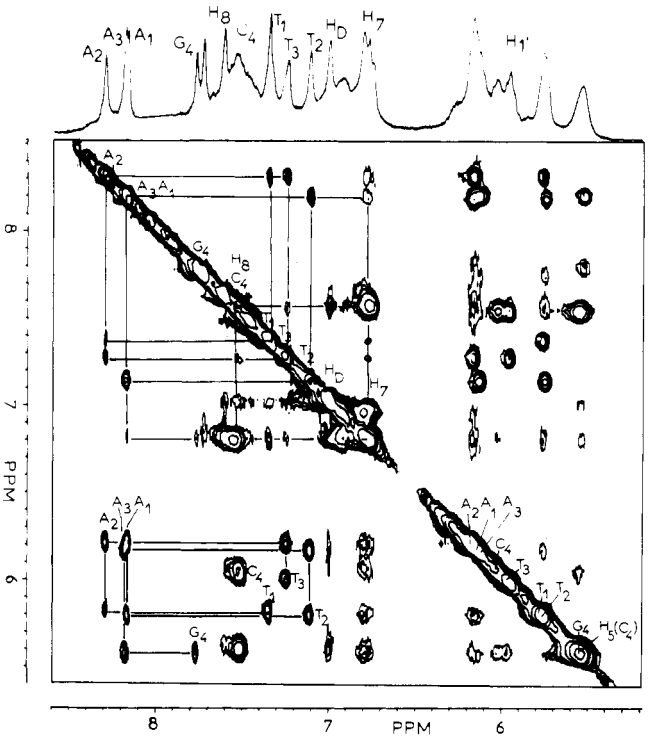

FIGURE 3: Expansion of the aromatic to $H_{1'}$ proton region of the NOESY spectrum of d(TATC)m_5Acr + d(GATA) at 21 °C in 0.2 M NaCl, pH 7.0. The sequential assignment of the two strands is indicated with a solid line. Cross peaks between protons of neighboring bases and between some aromatic protons of bases and acridine are also shown by a solid line. The labeled connectivities correspond to the observed cross peak between the H_8 or H_6 resonances of the base and their own $H_{1'}$ resonances. The other cross peaks (in the region 5.5–6.2 ppm) correspond to the connectivities with the $H_{1'}$ resonances of their 5′-neighbor. The connectivities between the H_6 or H_8 resonances and the protons of the dye (H_D, H_7, and H_8) are shown in the regions 6.6–8.4 and 6.6–8.4 ppm. Some weak connectivities between the neighbor $H_{1'}$ resonances are also shown in the regions 5.5–6.2 and 5.5–6.2 ppm.

Figures 3–5 present three parts of the NOESY map of the mixture d(TATC)m_5Acr + d(GATA) (7 mM:7 mM) at 21 °C. Figure 3 shows six double spots (8.30, 8.19, 8.17, 7.52, 7.25, and 7.12 ppm) and two single spots (7.78 and 7.35 ppm) between the H_8 or H_6 resonances and the $H_{1'}$ or H_5 resonances. The COSY DQF map (data not shown here) was used to locate the connectivity of the H_6 and H_5 protons of the cytosine C_4 at 7.52–5.55 ppm. Therefore, among the eight H_8 or H_6 resonances of the bases, five resonances present two NOE effects with the $H_{1'}$ resonances of the sugars (5.5–6.2 ppm), and three resonances present only one NOE effect in the range 5.5–6.2 ppm.

Since the sequences d(TATC) and d(GATA) contain only one guanine, the H_8 (G_4) resonance at 7.78 ppm was recognized as a singlet resonance well separated from the other H_8 adenine resonances; this is also the H_8 resonance that presents only one NOE effect with the $H_{1'}$ resonances (guanine G_4 has no 5′-neighbor base). By use of the fact that each H_8 or H_6 proton is close to its own $H_{1'}$ proton and to the $H_{1'}$ proton of one of its neighbor bases, the H_8/H_6 protons of d(GATA) were sequentially assigned. By the same procedure, starting from the 5′ residue (T_1) whose H_6 resonance presents only one NOE effect with the $H_{1'}$ protons, the four H_8/H_6 protons of d(TATC) were assigned (Figure 3). The same procedure was repeated by using the NOE effects between the aromatic H_8/H_6 protons and the $H_{2'}/H_{2''}$ protons of sugars (Figure 4). The assignment of the 2′/2″ sugar protons was confirmed by using the NOE effects observed between the $H_{1'}$ and the

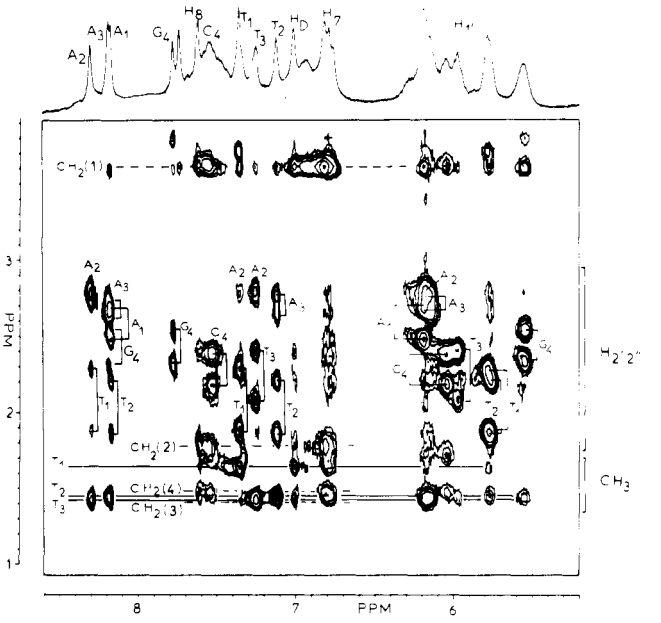

FIGURE 4: Expansion of the aromatic to $H_{2'}/H_{2''}$ proton region of the NOESY spectrum of d(TATC)m_5Acr + d(GATA) at 21 °C in 0.2 M NaCl, pH 7.0. The frequency of the resonances of the base aromatic protons is given as well as the assignment of all $H_{2'}$–$H_{2''}$ resonance lines. T_1, T_2, and T_3 and CH_2 show the position of the methyl group of thymines and the methylene chain: PO_4–CH_2-(5)–CH_2(4)–CH_2(3)–CH_2(2)–CH_2(1)–Acr. Each H_8 or H_6 resonance (except T_3, G_4, and C_4) shows a connectivity with its own $H_{2'}$–$H_{2''}$ resonances and with the $H_{2'}$–$H_{2''}$ resonances of their 5′-neighbor. The connectivities observed between the $H_{1'}$ and the $H_{2'}$–$H_{2''}$ resonances are in agreement with the connectivities observed on the COSY map (Figure 6).

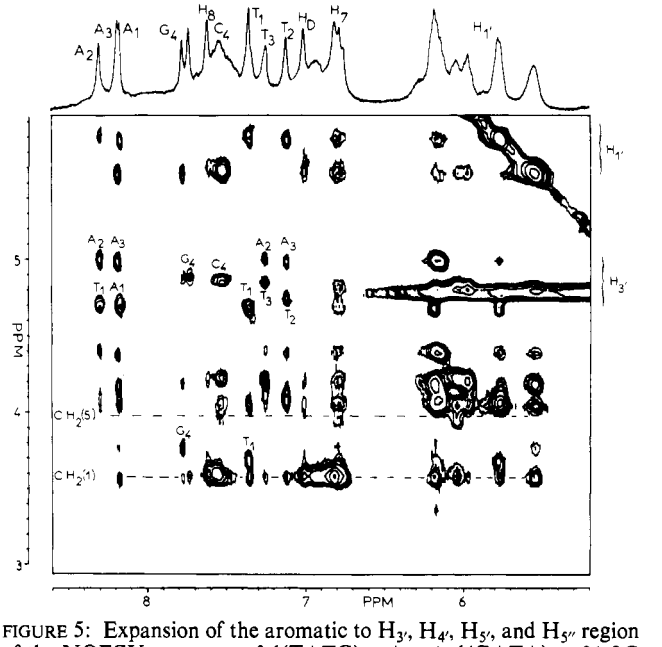

FIGURE 5: Expansion of the aromatic to $H_{3'}$, $H_{4'}$, $H_{5'}$, and $H_{5''}$ region of the NOESY spectrum of d(TATC)m_5Acr + d(GATA) at 21 °C in 0.2 M NaCl, pH 7.0. The frequency of the resonances of the base aromatic protons is given as well as the assignment of the $H_{3'}$ resonances. The positions of CH_2(1), CH_2(5), and $H_{5'/5''}$ of G_4 and T_1 are also given.

$H_{2'}/H_{2''}$ protons belonging to the same residue and the COSY DQF spectra (Figure 6). The NOE effects between H_5 and H_6 of cytosine and between CH_3 and H_6 of thymine were used for the assignment of H_5 (C) and CH_3 (T) (Figures 3 and 4). Moreover the observed interbase NOE effects H_8 (A_2)–H_6 (T_1), H_8 (A_2)–H_6 (T_3), H_8 (A_3)–H_6 (T_2), and H_6 (C_4)–H_6 (T_3) confirm these sequential assignments (Figure 3).

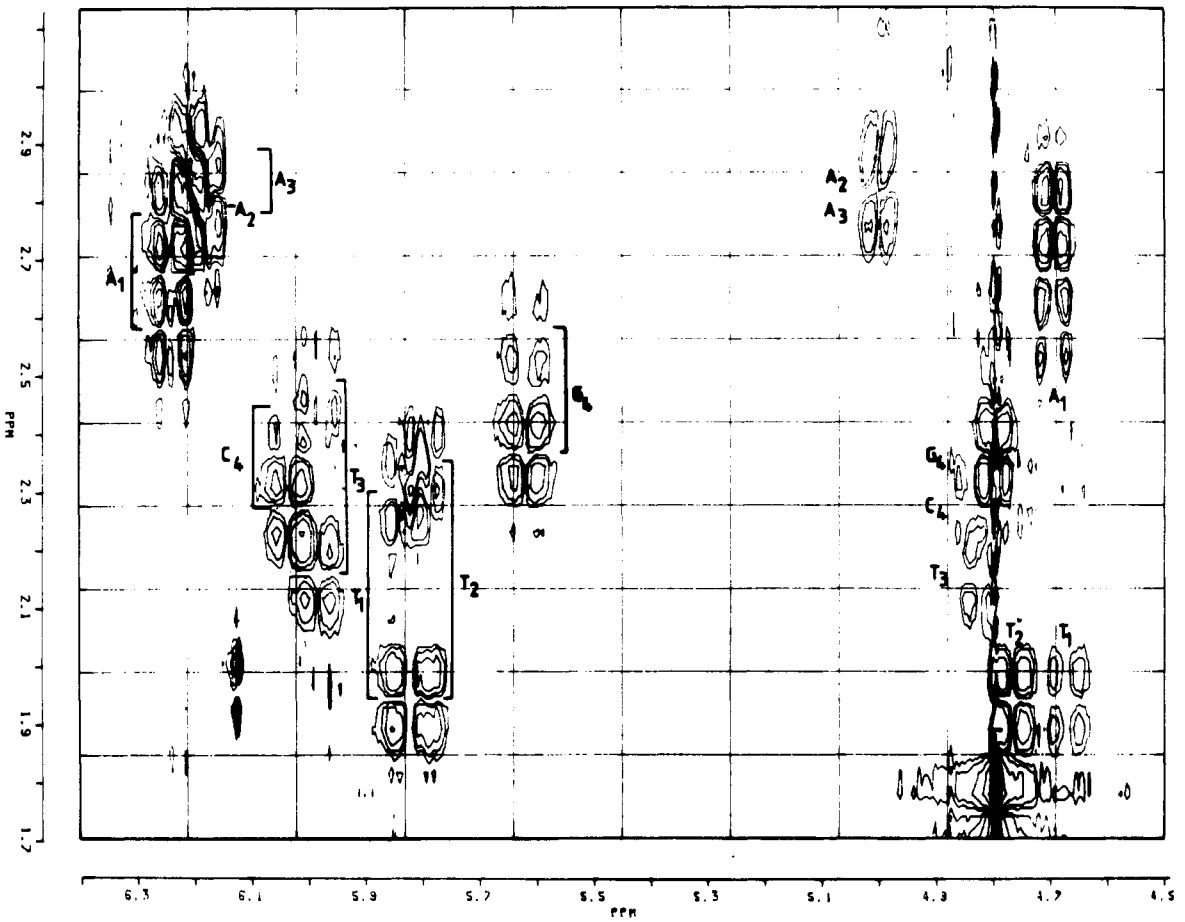

FIGURE 6: Expansion of the sugar proton region ($H_{1'}$, $H_{2'}$, $H_{2''}$, $H_{3'}$) of the COSY DQF spectrum of the mixture d(TATC)m_5Acr + d(GATA) (3 mM:3 mM) at 21 °C in 0.2 M NaCl, pH 7.0. The 2″ resonances were assigned by their weak cross peak with the $H_{1'}$ resonances and by their cross peak with the $H_{3'}$ proton. It is worth noting that all the 2″ resonances [except the degenerate $H_{2'}/H_{2''}$ (A_2) resonances] are located at lower field than their homologous 2′ resonances and that the 2′ resonances did not present connectivities with the $H_{3'}$ resonances (except for A_1).

Table I: Computed Distances between the H_8 or H_6 Proton of a Base (n) and the Protons of Its 5′-Neighbor ($n - 1$) or Its 3′-Neighbor ($n + 1$) Nucleotide for DNA of Type B Right, Type A Right, and Type B Left[a]

	H_8 (A)	H_8 (G)	H_6 (T)	H_6 (C)	H_5 (C)	CH_3 (T)	$H_{1'}$	$H_{2'}$	$H_{2''}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{5''}$
						DNA Type B Right (Premilat et al., 1983)							
$n - 1$	4.94	4.90	4.78	4.76	6.18	7.03	3.63	3.20	1.84	4.03	5.66	7.17	6.18
n							3.88	2.43	3.89	5.15	4.96	5.13	3.35
$n + 1$	4.94	4.98	5.20	5.22	3.83	3.13	7.65	7.31	8.63	9.58	8.34	7.81	6.56
						DNA Type A Right (Premilat et al., 1983)							
$n - 1$	4.70	4.69	4.67	4.67	5.21	5.95	4.39	1.97	3.79	3.68	5.25	6.44	5.64
n							3.58	4.26	4.84	4.81	4.49	4.63	2.84
$n + 1$	4.70	4.74	4.90	4.93	4.06	3.37	7.60	8.90	9.35	9.35	8.58	7.96	6.22
						DNA Type B Left (Gupta et al., 1980)							
$n - 1$	5.06	5.10	5.21	5.24	3.77	2.97	8.04	6.75	8.02	9.70	9.20	9.35	7.84
n							3.62	3.65	4.68	5.61	4.47	5.32	3.83
$n + 1$	5.06	5.02	4.93	4.92	6.45	7.17	2.39	5.33	4.55	4.33	3.51	5.80	5.63

[a] The distances were computed by assuming all the C–H distances equal to 1 Å and by taking the proposed coordinates for the three families of DNA: A and B right (Premilat et al., 1982); B left (Gupta et al., 1980).

Conformation of Duplex. Table I shows the interatomic distances computed for right- and left-handed models of B DNA and A DNA. It is important to note that in right-handed DNA the H_8 or H_6 proton of a base is close to the $H_{1'}$ proton sugar of its 5′-neighboring nucleotide and far from all the sugar protons of its 3′-neighboring nucleotide. In contrast, the opposite situation is observed in left-handed DNA (Table I).

From the assignment made above and the observation that the nearest $H_{1'}$ proton of each H_8 or H_6 proton always belonged to the 5′-neighboring nucleotide, we conclude that the duplex adopts a right-handed helical structure. In the isolated single-strand d(TATC)m_5Acr and d(GATA), we observed NOE effects only between the H_8 or H_6 protons and their own $H_{1'}$ or $H_{2'}/H_{2''}$ protons. We conclude that in these single-stranded oligonucleotides the bases are far from the other bases or the neighboring sugars.

Information about sugar geometry can be obtained from the scalar coupling constants observed between the protons $H_{1'}$, $H_{2'}$, $H_{2''}$, and $H_{3'}$. In a pure 2′-endo geometry, $J_{1'2'} = 10.1$ Hz, $J_{1'2''} = 5.5$ Hz, $J_{2'3'} = 5.5$ Hz, and $J_{2''3'} = 0.1$ Hz while in a pure 3′-endo geometry $J_{1'2'} = 0.1$ Hz, $J_{1'2''} = 7.4$ Hz, $J_{2'3'}$

Table II: Assignment of Proton Resonances in the Mini Double Helix d(TATC)m_5Acr + d(GATA) (7 mM:7 mM) in 0.2 M NaCl at 21 °C

	H_8	H_6	H_5	CH_3	H_2	$H_{1'}$	$H_{2'}$	$H_{2''}$	$H_{3'}$	$H_{4'}$
T_1		7.35		1 70		5.78	1.93	2.34	4.72	4.06
A_2	8.3				7.58	6.19	2.84	2.84	5.02	4.37
T_3		7.25		1.48		5.97	2.13	2.46	4.88	4.20
C_4		7.52	5.55			6.05	2.25	2.44	4.89	4.21
G_4	7.78					5.54	2.50	2.59	4.91	4.19
A_3	8.19				7.74[a]	6.19	2.71	2.82	5.00	4.37
T_2		7.12		1.49		5.76	1.92	2.26	4.78	4.10
A_1	8.17				7.62[a]	6.16			4.74	4.19

[a] The absence of NOE connectivities involving the H_2 resonances of adenine A_1 and A_3 precluded their separate assignment.

= 4.9 Hz, and $J_{2''3'}$ = 11.0 Hz (Sarma, 1980).

To obtain a good resolution for a weak coupling requires a longer acquisition time than for a larger coupling. In fact, the relaxation processes as well as the limitations of computer memory and instrument time artificially decrease the intensity of the cross peaks corresponding to weak coupling constants and allow us to differentiate between the large and small coupling constants by simple analysis of the intensity of the connectivities in a COSY map. In these conditions of low digital resolution, we can predict that the system 1', 2', 2'', 3' will present three cross peaks with very different characteristics for 2'-endo or 3'-endo sugar conformations. In the 2'-endo conformations, the $H_{1'}$ proton will present two cross peaks with the $H_{2'}$ and $H_{2''}$ protons ($H_{2'} > H_{2''}$, $J_{1'2'}$ = 10.1 Hz, $J_{1'2''}$ = 5.5 Hz), and the $H_{3'}$ proton will present only one cross peak with the $H_{2'}$ proton ($J_{2'3'}$ = 5.5 Hz, $J_{2''3'}$ = 0.1 Hz). In the 3'-endo conformation, the $H_{1'}$ protons will present only one cross peak (or additionally a very weak second cross peak) with $H_{2''}$ protons ($J_{1'2'}$ = 0.1 Hz, $J_{1'2''}$ = 7.4 Hz), and the $H_{3'}$ protons will present two cross peaks with $H_{2'}/H_{2''}$ protons ($J_{2'3'}$ = 4.9 Hz, $J_{2''3'}$ = 11 Hz).

An analysis of the COSY DQF map (Figure 6) shows that except for A_2 whose $H_{2'}$ and $H_{2''}$ resonances were quasi-degenerated (as for a 2'-endo conformation) each $H_{2'}$ proton showed two connectivities of slightly different intensity with the $H_{2'}/H_{2''}$ protons. Moreover, the $H_{2''}$ resonances (corresponding to the weaker connectivity $H_{1'}$–$H_{2'}/H_{2''}$) were connected to the $H_{3'}$ resonance while the $H_{2'}$ resonances were not connected (except for A_1 where the $H_{2'}$–$H_{3'}$ and the $H_{2''}$–$H_{3'}$ connectivities were observed). Therefore, we conclude that the sugar conformation is predominantly 2'-endo in the duplex structure. This conclusion is supported by the fact that the sums of the coupling constants $J_{1'2'} + J_{1'2''}$ measured directly on the row of the COSY data matrix after zero filling at 16 K were all in the range 12.8–14.4 Hz. All the $H_{2'}$ resonances were observed at higher field (except the degenerate resonances $H_{2'}$ and $H_{2''}$ of A_2) than the $H_{2''}$ resonances (Table II).

At 21 °C, only part of the duplex was formed between single strands d(TATC)m_5Acr and d(GATA) (Figure 2). At lower temperature, the resonance lines were broadened due to self-association. Therefore, it was not possible to quantify the NOE cross peaks of the isolated duplex form. Nevertheless, the NOESY map obtained for short mixing times (τ_m = 150–200 ms) shows that the connectivities of H_6 or H_8 with the $H_{2''}$ proton belonging to the 5'-neighboring nucleotide [H_6 or H_8 (i)–$H_{2''}$ (i − 1)] are stronger than the connectivities H_6 or H_8 (i)–$H_{2'}$ (i), –$H_{2''}$ (i), and –$H_{2'}$ (i − 1). These characteristics are in agreement with a B conformation where the order of distances with H_6 or H_8 is $H_{2''}$ (i − 1) > $H_{2'}$ (i) > $H_{2'}$ (i − 1) > $H_{1'}$ (i − 1) ≃ $H_{1'}$ (i) ≃ $H_{2''}$ (i) rather than an A conformation where $H_{2'}$ (i − 1) ≫ $H_{1'}$ (i) ≃ $H_{2''}$ (i − 1) > $H_{2'}$ (i) ≃ $H_{1'}$ (i − 1) > $H_{2''}$ (i).

Moreover, in the B conformation protons H_6 or H_8 are closer to the H_5 proton of the 3'-neighboring cytosine (n + 1) or the CH_3 of the 3'-neighboring thymine (3.8–3.6 Å) than the corresponding proton of the 5'-neighboring base (6–7 Å) whose NOE connection cannot be observed [see, for example, the observed NOE effects in d(ATATGCATAT), Feigon et al., 1983]. As a matter of fact, a NOE effect was observed between the protons H_8 (A_2) and CH_3 (T_3) and between H_8 (A_3) and CH_3 (T_2) while no connection was observed with the methyl group of the initial residue T_1 and with any of the H_6 or H_8 protons.

Some weaker NOE effects were also observed at 15 °C between the protons H_6 or H_8 of the neighboring bases: H_8 (G_4)–H_8 (A_3), H_8 (A_3)–H_6 (T_2), H_6 (T_2)–H_8 (A_2), H_6 (T_1)–H_8 (A_2), H_8 (A_2)–H_6 (T_3), and H_6 (T_3)–H_6 (C_4). These weak connections correspond to computed distances of about 5 Å in the classical A or B conformation. All these data are indicative of the formation of a duplex structure, with stacked bases in a conformation belonging to the B family with 2'-endo sugars.

Intercalation of Acridine Derivative. The H_7 and H_8 protons of the acridine derivative were assigned by use of their connectivities observed on COSY and NOESY spectra and the assignment already made for the same acridine derivative linked to an oligothymidylate (Lancelot et al., 1985). The so-called H_D protons correspond to the H_1, H_3, H_4, and H_5 protons, which are overlapped and/or strongly coupled and which could not be individually assigned in the complex. The characteristic singlet of the 2-methoxy group was found at about 3.6 ppm. The protons of the methylene chain were assigned from the COSY DQF spectra assuming that $CH_2(1)$ rather than $CH_2(5)$ should present NOE connectivities with the protons of the acridine ring (Figure 5).

Figure 3 shows the NOE effects observed between the H_7 proton of acridine and the H_6 protons of thymines T_1 and T_3, the H_8 proton of guanine G_4, and the H_5 proton of cytosine C_4. They provide direct proof of the proximity of the dye with the base pairs. Other NOE effects were observed between proton H_7 of the acridine and the resonances lines at 7.5 and 1.5 ppm. Although these positions correspond to the protons H_6 of cytosine C_4 and the methyl groups of thymines T_1, T_2, and T_3, their overlap with the protons H_8 (Acr) and the methylene groups prevented us from assigning the corresponding connectivies. The other protons of acridine, called H_D, also presented some NOE effects with cytosine C_4 or sugar protons [H_D–H_6 (C_4), $H_{1'}$ (A_1 or A_2 or A_3, C_4, T_1 or T_2, G_4)–H_7 and H_D (Figure 3)] while the 2-methoxy group never exhibited any NOE cross peak with the protons of the nucleotides. The methylene groups $CH_2(1)$ showed several NOE connectivities with protons of the nucleotides [H_8 (A_1), H_8 (G_4), H_6 (T_3), $H_{1'}$ (A_1 or A_2 or A_3, C_4, T_1 or T_2, G_4)] or with the other methylene groups $CH_2(2)$ and $CH_2(3)$.

The protons $CH_2(2)$ were found close to the H_6 of cytosine C_4 while the group $CH_2(5)$ (which is linked to the phosphate of nucleotide C_4) did not exhibit any NOE effect with the nucleotides or the acridine ring. The H_2 resonances of adenine,

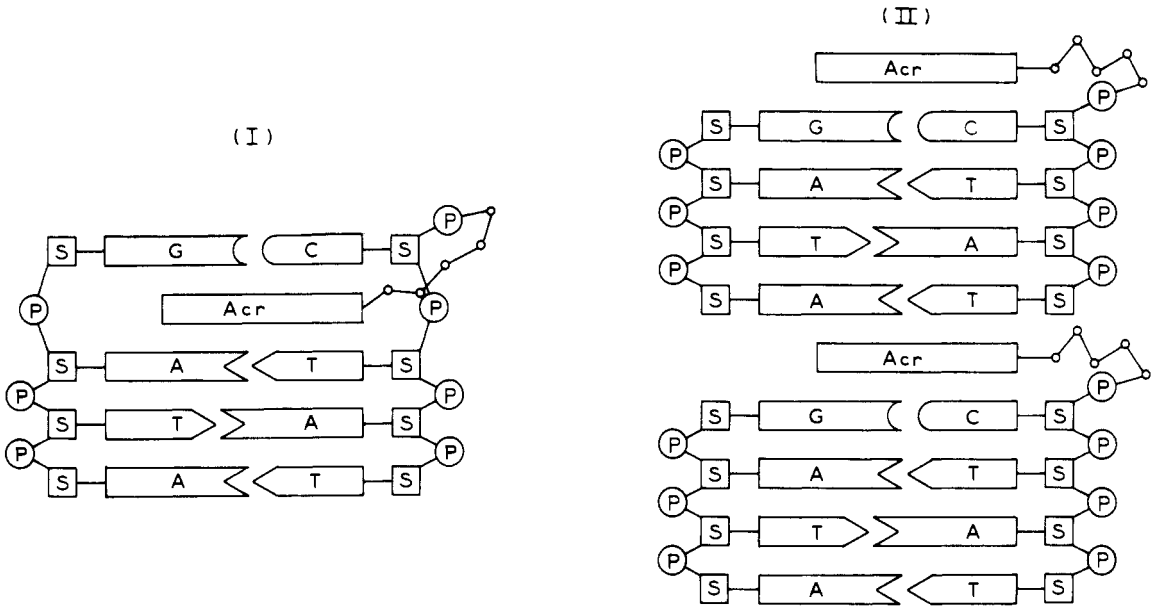

FIGURE 7: Model of equilibrium between two conformations where the acridine dye is intercalated between the G_4-C_4 and A_3-T_3 bases pairs (I) or between two duplexes (II). These two conformations are proposed by using the observed NOE effects between the dye and the nucleotides (see text). In model II, the duplex can adopt several geometries depending on the twist around the vertical axis of the helix. Model II, where the two duplexes adopt a head to tail conformation, may also exist in solution (see text).

assigned by their temperature dependence (Figure 2) and their long T_1 relaxation time showed a weak NOE effect with the protons H_D (Acr), H_7 (Acr), and $CH_2(1)$.

Discussion

Our data unambiguously show that in aqueous solution and below 25 °C the oligodeoxynucleotides d(GATA) and d(TATC)m_5Acr form a mini double helix that adopts a B-type conformation. Structural informations from the relative intensities of COSY and NOESY maps revealed that the deoxyriboses adopt preferentially a 2′-endo conformation. NOE connectivities were observed not only between the dye (or the methylene chain) and the nearest base pairs C_4-G_4 and T_3-A_3 but also between the dye and the distant base pair T_1-A_1. Although it can exist as a family of aggregates with such a geometry that they do not contribute to the observed cross peaks in NOESY maps, we can conclude the presence of an equilibrium between two conformational families (Figure 7) at least. In the first conformation, the acridine derivative is intercalated between base pairs C_4-G_4 and T_3-A_3 while in the second conformation the acridine is stacked outside of the duplex, on top of base pair C_4-G_4 and sandwiched between the C_4-G_4 base pair of its own duplex and the T_1-A_1 base pair belonging to a second duplex. This last model is in agreement with the observation that below 20 °C all the resonance lines were drastically broadened, corresponding to the formation of aggregated species that have longer correlation times. The first model is in agreement with the fact that NOE effects have not been observed between $H_{1'}$ or $H_{2'}/H_{2''}$ of T(3) and H_6 of C(4), due to the intercalation of the acridine moiety. This fact has already been observed between the internal guanine and cytosine in the double helices d(AGCT) and d(ATGCAT) where actinomycin D was intercalated between the central base pairs G-C and C-G (Reid et al., 1983; Brown et al., 1984).

Building of molecular Corey–Pauling–Koltren (CPK) models showed that a linker with five methylene groups allowed either stacking of the acridine rings on top of the C_4-G_4 base pair (configuration II) or intercalation between base pairs T_3-A_3 and C_4-G_4 (configuration I). In both cases, the intercalating ring must enter via the small groove of the double helix. This structure was proved by the observed NOE effect between the protons of acridine and the H_2 protons of adenine, which are located in the minor groove.

For each configuration (I or II), the acridine ring can adopt two positions, depending of the rotation of the acridine moiety aroung the C_9–NH bond. In both positions the methylene groups were found close to protons of sugars belonging to the same strand as C_4. The observed connectivities between the methylene group $CH_2(1)$ and the $H_{1'}$ protons of A_1, T_1, and G_4 are indicative of a short average distance between the methylene group $CH_2(1)$ and bases, which can extend as far as the initial and the terminal residues (A_1 and G_4) of the complementary strand. CPK models provide evidence that such proximities cannot exist in only one conformation. These connectivities are indicative of several possible geometries of stacking between duplexes: the angle between the axis of the last pair C_4-G_4 and that of the first pair T_1-A_1 of the neighbor duplex can take several values corresponding to different energy minima in conformation II. Further, a duplex of configuration I can be stacked with a group of duplexes of configuration II in the same sense 5′ → 3′ or head to tail in the sense 3′ → 5′.

It is worth noting that although configuration I is not concentration dependent and although configuration II is, we did not observe important differences in NOE connectivities between samples at 3 and 7 mM (strand concentration). In several of the CPK models, the methoxy group was found close to protons of the methylene groups or to sugar protons of cytosine C_4, but we never observed such a connectivity in the NOESY map. The free rotation of the OCH_3 group around the C_2–O bond, which decreases the correlation time of the CH_3 group and allows only a small percent of favorable positions, might induce a strong decrease of the average NOE intensity. The NOESY data do not exclude other structures that are not expected to lead to typical connectivities between the dye and the bases or the sugars. For example, the aromatic dye that bears a positive charge at pH 7.0 can electrostaticly bind a phosphate group of the complementary sequences. This location of the aromatic dye is quite possible in CPK models of the complex.

It is worth noting that although configurations I and II predict the formation of base pairs, we never observed the NH

resonances of the base pairs in aqueous solution (90% H_2O, 10% D_2O). At low temperature (10 °C), line broadening due to self-association of the complexes prevented us from obtaining reliable data on NH resonances.

At higher temperature (>20 °C) when line broadening was no longer the limiting parameter, the complex was in too fast of an exchange with the separated molecules, and rapid exchange of water with the NH protons of the bases could not be avoided. Such an observation was previously made for short duplexes such as $(Tp)_4m_5Acr + (Ap)_3A$ (Lancelot et al., 1985). The observation of the NH resonances in oligonucleotides linked to intercalating dye requires the choice of sequences where the formation of isolated duplexes is favored as compared to aggregates. The NH resonances have been observed for longer sequences where at 30 °C aggregation is weak (G. Lancelot, unpublished results).

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