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Conformations of Two Duplex Forms of d(TCGA) in Slow-Exchange Equilibrium Characterized by NMR†

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ABSTRACT: Two conformations adopted by the tetranucleoside triphosphate d(TCGA) in aqueous solution are in slow-exchange equilibrium on the NMR time scale. ^1H and ^{31}P NMR spectra obtained at temperatures below 25 °C contain two sets of signals that vary in relative proportions with changing temperature. High-field NMR techniques allow the conformations of these species to be examined. Both forms are right-handed double-helical structures, and their interconversion does not involve a single-stranded species since transfer of saturation is observed between corresponding imino protons held in the base pairs of each duplex. The form that predominates at higher temperatures resembles B-DNA, but the other, while of similar conformation at the ends of the molecule, is distorted at the C-G step. Shearing at the center of the duplex results in interstrand stacking of the two cytosines in a way that is reminiscent of Z-DNA. Distances between nonexchangeable protons in this model are consistent with nuclear Overhauser effects observed for resonances of the low-temperature form, while the ^1H NMR spectrum shows cytidine H-2′ resonances at unusually high field. The relative stabilities of the two forms are discussed in terms of base stacking and hydration, but the origin of the high activation energy for interconversion implicit in the slow-exchange rate is unclear. The conformation of the low-temperature form may represent a sequence-dependent structural feature important in natural DNA, although somewhat fortuitously exemplified by this tetramer. The suggested involvement in correct nucleosome phasing of the pentamer d(TTCGA), present in some eukaryotic genes, is noted.

The double-helical structures formed by nucleic acids are classically built up as a succession of interchangeable nucleotide pairs. Despite the potential of this arrangement for regularity, high-resolution, single crystal X-ray analysis of oligonucleotide fragments of A- and B-DNA (Drew et al., 1980; Viswamitra et al., 1978; Conner et al., 1982; Wang et al., 1982; Shakked et al., 1983) have shown considerable variation from the classical geometries derived by using data from polymeric DNA. In B-type structures, this variation has been correlated with the properties of polynucleotides in solution (Klug et al., 1979; Lomonossoff et al., 1981) and is

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attributed to optimization of base pair stacking overlaps (Klug et al., 1979) and accommodation of steric repulsion between neighboring base pairs (Calladine, 1982). Characteristic conformations associated with particular sequences of nucleotides in DNA introduce an additional dimension to the information content and can provide features that may, for example, be exploited in specific or selective interactions with binding proteins.

Short stretches of natural DNA implicated in various aspects of genetic control frequently are found to possess more or less homologous sequences. Details of the local structure in polymeric DNA that may be important in understanding its behavior in molecular terms are intractable to high-resolution study; such information is at present only accessible by using model systems. By this approach, single crystal X-ray studies have been instrumental in resolving some structural problems associated with polymeric DNA (Wang et al., 1979; Klug et al., 1979) as well as drawing attention to the potential for sequence-dependent conformation.

NMR spectroscopy has been widely applied to oligonucleotides in solution. It is a source of detailed structural information but unlike X-ray analysis is free from constraints, both molecular and experimental, imposed by the crystalline state. Recent developments, particularly the use of the nuclear Overhauser effect (NOE), make the technique a powerful structural probe. The NOE is a consequence of dipolar relaxation between nuclei (Noggle & Schirmer, 1971); it is measured as the change in integrated intensity of a resonance when the magnetization of a second nucleus which contributes relaxation to it is perturbed by irradiation. The rate of buildup of the NOE is proportional to the inverse sixth power of the distance separating the two nuclei. Structurally useful effects can be measured for protons up to ca. 4 Å apart.

NOEs are observed between nonexchangeable protons on adjacent nucleotide residues in a helical structure. They enable extensive and reliable assignment of the spectrum to be made in a sequential manner as well as providing a basis for conformational arguments (Reid et al., 1983a,c; Feigon et al., 1982; Scheek et al., 1983; Hare et al., 1983; Wemmer et al., 1984; Haasnoot et al., 1983; Cheng et al., 1984; Weiss et al., 1984.

Much of the information obtained with NMR has tended toward the confirmatory. We report here, however, that the tetranucleoside triphosphate d(TCGA) exists in two duplex forms that interconvert slowly on the NMR time scale. Using NOEs in combination with other NMR techniques, we have been able to show that one form, which predominates at higher temperatures, resembles B-DNA, while in the second, the helix is distorted by shearing at the C-G junction, producing base-pair overlaps considerably different from those normally encountered in double-helical DNA. This observation may have some relevance to the suggested involvement of the pentanucleotide d(TTCGA), located at the origin of nucleosome assembly of eukaryotic tRNA genes, in nucleosome phasing (Richmond et al., 1983).

Materials and Methods

Nucleotide. The tetranucleoside triphosphate d(TCGA) was synthesized by using the solid-phase triester method (Gait et al., 1982). It was purified by DEAE-cellulose chromatography and converted to its ammonium salt by passage through a column of ammonium Dowex AG50-X8.

NMR Spectroscopy. Nonexchangeable ^1H and ^{31}P NMR spectra were obtained by using a Bruker WH-400 interfaced to an Aspect 2000 computer and operating with quadrature detection in the Fourier transform mode. NOE and saturation transfer difference spectra resulted from subtraction of accumulated off-resonance (control) and on-resonance spectra acquired alternately. Irradiation levels were adjusted in accordance with selectivity requirements. Irradiation times varied from 0.1 to 0.5 s, and a delay of 2 ms was inserted between the irradiation and observation pulses. A 90° pulse of 7 μs and relaxation delay of 3 s were used. NOE magnitudes were estimated by comparing the intensities of the irradiated signal with those of other signals showing NOEs. For the observation of nonexchangeable protons in ^1H spectra, 1.5 mg of nucleotide was lyophilized twice from D_2O (or deuterated 0.1 M sodium phosphate buffer) and finally dissolved in 0.4 mL of 99.996% isotopically enriched D_2O (Aldrich). ^{31}P spectra were recorded with 8 mg of nucleotide in 1.5 mL of D_2O contained in a 10-mm outer diameter NMR tube. A 90° pulse of 22 μs was used followed by a total relaxation delay time of 3 s. All samples gave pH meter readings between 6.8 and 7.2. When necessary, pH was adjusted by the addition of aliquots of DCl or NaOD.

Spectra of exchangeable protons were obtained on a Bruker AM500 operating out of the quadrature mode. The sample (1.5 mg) was dissolved in H_2O/D_2O (95:5) and the water resonance suppressed by using the 1–2–1 pulse train of Sklenář & Starčuk (1982) without phase alternation in the second pulse. Best results were achieved by placing the carrier frequency downfield of the H_2O signal and switching the transmitter and receiver phases by 180° on alternate scans.

Model Building. Nicholson "Labquip" ball and stick, Kendrew, and CPK space-filling models were used in various stages of the structural work.

Results

The ^1H NMR spectrum of d(TCGA) in D_2O and in deuterated phosphate buffer at elevated temperatures (>50 °C) is typical of that of a single-stranded oligonucleotide: the six aromatic and four anomeric proton signals are sharp and well resolved. As the temperature is lowered, changes occur of a kind usually interpreted as being due to duplex formation: many signals move to higher field under the influence of ring-current shielding effects as base stacking increases. Below 28 °C this trend continues, but a second set of signals appears, which increases in relative proportion as the temperature is lowered. At 10 °C and at an oligonucleotide concentration of 1.5 mg in 0.4 mL the two superimposed spectra are of equal intensity (Figure 1a), and in D_2O at 1 °C the form evident only at lower temperatures is clearly predominant (Figure 1b). In buffer, however, both spectra are considerably broadened at this temperature. At any temperature raising the concentration of nucleotide tends to increase slightly the relative amount of the low-temperature form, although this dependence has not been examined in detail. The spectrum of the imino protons in H_2O (Figure 2), and the ^{31}P spectrum (Figure 3) show similar behavior.

Structures that contribute to spectra of double-stranded oligonucleotides, are almost invariably found to be in fast exchange on the NMR time scale, in this case requiring a rate of interconversion greater than 1000 Hz. The behavior of d(TCGA) is therefore strikingly anomalous.

The chemical shifts and line widths in the spectrum of the high-temperature form, below about 10 °C, are consistent with a duplex structure. This conclusion is supported by the fact that protons in the base pairs are in slow exchange with bulk water and can be observed. A duplex structure is also indicated for the low-temperature form, with the exception of some remarkably shifted resonances. One conspicuous feature of the spectrum is a broad signal at δ 1.0 [relative to internal

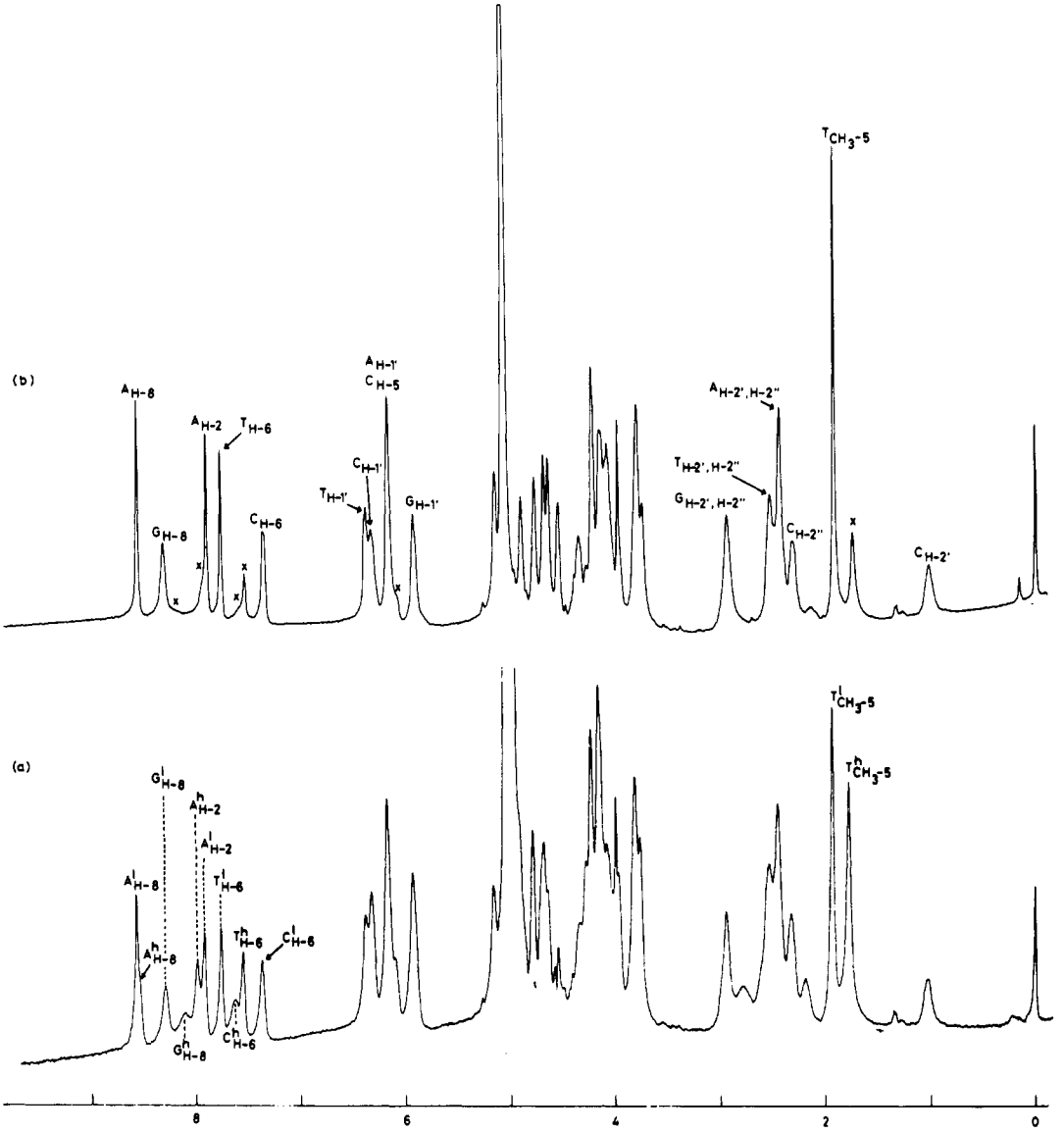

FIGURE 1: ^1H NMR spectrum of d(TCGA) in D_2O at (a) 10 and (b) 2 °C. Superscripts h and l denote resonances assigned to the high- and low-temperature forms, respectively. Peaks marked X in (b) are due to the high-temperature form. Chemical shifts are measured in ppm downfield of DSS; 100 scans were accumulated by using 7-μs 90° pulses followed by a total delay of 3 s, sweep width of 4000 Hz, and 8K digitization.

4,4-dimethyl-4-silapentanesulfonate (DSS)] assigned to a 2′-methylene proton; the strong upfield shift compared to the high-temperature form indicates a structure considerably different from the latter as well as other conventional duplex conformations.

In order to investigate this apparently novel structure, a series of NOE experiments was performed at 1 °C. NOEs between nonexchangeable protons of DNA oligomer duplexes of right-handed B-type geometry are fairly well understood both qualitatively and quantitatively (Reid et al., 1983a,b). Irradiation of a purine H-8 (or pyrimidine H-6) resonance results in NOEs to protons in the same as well as in adjacent nucleotide residues. In the same residue, there is a strong NOE to the nearer of the C-2′ methylene protons (H-2′), while effects to the more distant H-2″ and H-1′ are considerably weaker. Interresidue effects are characteristically different for 3′ and 5′ neighbors. In the 5′ direction, both H-2′ and H-2″ experience NOEs of magnitudes similar to the intraresidue effect to H-2′, while that to H-1′ is weaker and of the same order as that seen to H-1′ in the same residue. In the 3′ direction, thymine methyl protons and H-5 of cytosine show weak NOEs, while effects of similar magnitude are seen to purine H-8 (or pyrimidine H-6) of both flanking bases. Upon irradiation of the anomeric resonances, of all the H-2′ and H-2″ protons, only those of the same residue as the anomeric proton show NOEs.

We have used these observations for assigning the spectra of oligonucleotides and, by more quantitative application of NOE data, have been able to make deductions about the conformations of some oligomer duplexes (Reid et al., 1983a–c).

NOEs observed in the low-temperature form of d(TCGA) are summarized in Table I; Figure 4 shows some typical NOE difference spectra. In general the NOEs are consistent with a right-handed double helix according to the criteria outlined above and allow considerable assignment of the spectrum. There appear to be no major deviations in the overall pattern of NOEs observed from those consistent with a B-type double helix, and the sizes of the NOEs between anomeric and aromatic protons imply anti conformations about the glycosidic bonds. The relative magnitudes of some NOEs are, however, untypical. Irradiation of A_{H-8} (Figure 4a) produces the usual NOEs to $A_{H-1'}$ and $G_{H-1'}$, although relatively the latter is abnormally large, exceeding in magnitude the effect to $A_{H-2'}$

Table I: Percentage NOEs Observed in the Low-Temperature Form of d(TCGA) after 0.4-s Irradiation, Chosen As Providing the Best Compromise between the Necessity of Minimizing Spin Diffusion Effects and of Producing Changes of Readily Measurable Magnitude

proton irradiated		NOEs observed to residue															
		T				C					G			A			
residue	position	H-6	CH_3-5	H-1′	H-2′,H-2″	H-6	H-5	H-1′	H-2′	H-2″	H-8	H-1′	H-2′,H-2″	H-8	H-1′	H-2′,H-2″	H-2
T	H-6		16.5	5.5	23		3.6										
	CH_3-5	3.7															
	H-1′	3.6			12.0	5.3	9.5										
	H-2′,H-2″	16.1		24		1.9[b]											
C	H-6			6.9	*b*		30.7	7.5	21.6	16.3							
	H-5	0.8				7.0											
	H-1′					4.1			7.6	13.0	*b*						
	H-2′					35		17		64.5[a]	4.4						
	H-2″					13.8		21.3	36[a]		7.0		10.8				
G	H-8					2.7		1.0[b]	3.5	5.7		6.6	45.5				
	H-1′										3.9		22.6	7.1[c]			
	H-2′,H-2″										16.1	15.7		2.6			1.3
A	H-8											8.4[c]	2.9		2.2	2.8[b]	
	H-1′													2.3		33.8	0.4
	H-2′,H-2″													2.8[b]	12.0		

[a] These values are probably rendered unrealistic by spin-diffusion effects. [b] These NOEs are unexpectedly weak or absent. [c] These NOEs are unexpectedly relatively strong.

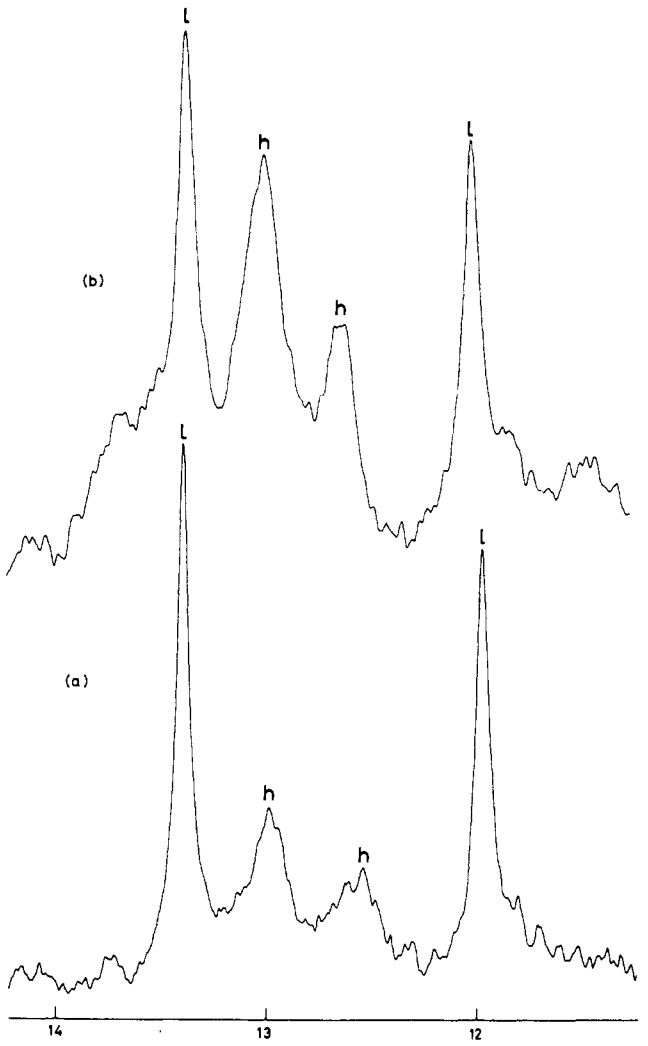

FIGURE 2: ^{1}H NMR spectrum (δ 11–14) of d(TCGA) in H_2O/D_2O (9:1) at (a) 2 and (b) 10 °C. Superscripts h and l denote resonances of high- and low-temperature forms, respectively.

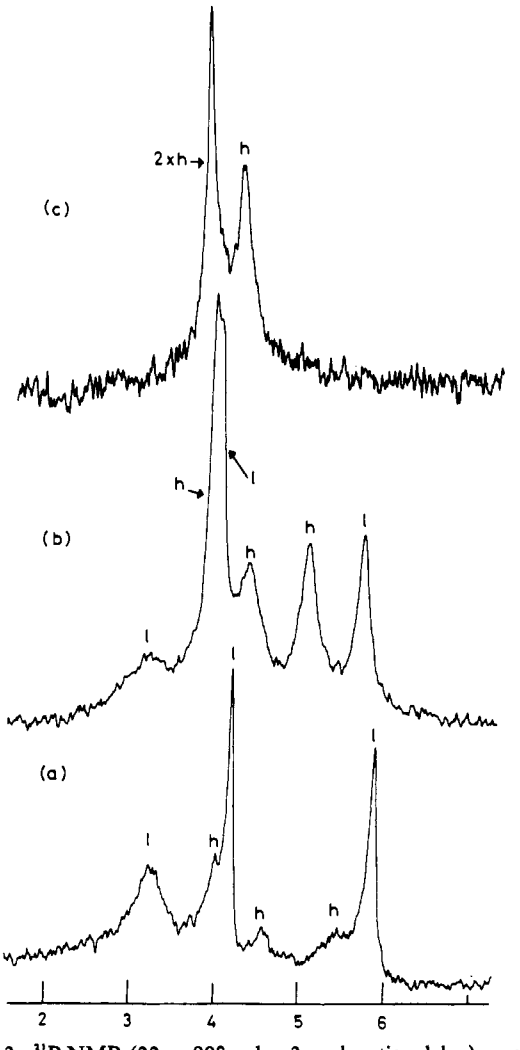

FIGURE 3: ^{31}P NMR (22-μs 90° pulse, 3-s relaxation delay) spectrum of d(TCGA) in D_2O at (a) 2, (b) 10, and (c) 20 °C. Chemical shifts are measured in ppm upfield of external 85% orthophosphoric acid.

and $A_{H\text{-}2''}$. The reciprocal NOE from $G_{H\text{-}1'}$ to $A_{H\text{-}8}$ is correspondingly large. On the other hand, the expected NOEs between $G_{H\text{-}8}$ and $C_{H\text{-}1'}$ are very small or absent as are those between $C_{H\text{-}6}$ and $T_{H\text{-}2',H2''}$ (Figure 4c).

Chemical shift differences between corresponding resonances in the high- and low-temperature forms are also a source of structural information. Between 0 and 8 °C little melting takes place; the chemical shifts of signals associated with each of the two forms remain virtually constant. With the spectrum of the low-temperature form assigned, saturation transfer techniques were used to extend these assignments to the high-temperature form (Figure 1a). Unfortunately, at tem-

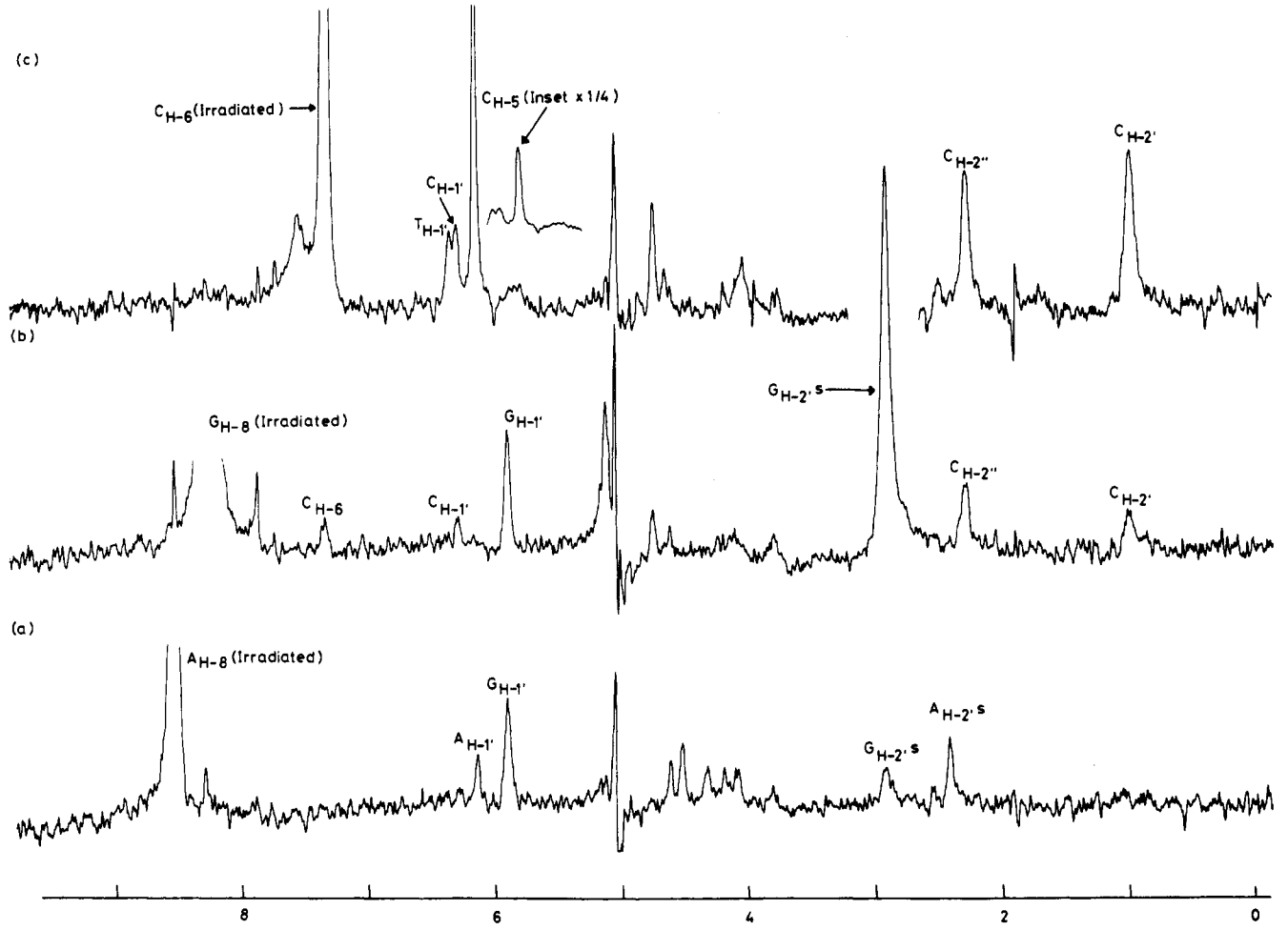

FIGURE 4: Typical ^1H NOE difference spectrum obtained after a 0.4-s irradiation at the position of the resonances indicated.

Table II: Summary of the Significant Chemical Shift Discrepancies between the Two Forms of d(TCGA) of the Assignable Protons[a]

upfield shifts	downfield shifts
A_{H-2} (+0.07)	G_{H-8} (−0.20)
C_{H-6} (+0.27)	T_{J-6} (−0.22)
$C_{H-2'}$ (+0.75)	T_{CH_3-5} (−0.15)
	$G_{H-2'}$ (~0.15)
	$G_{H-2''}$ (~0.15)

[a] The direction of the shift is with respect to a conversion from the high- to the low-temperature form. Thus, an upfield shift for C_{H-6} means that this proton is shielded in the low-temperature relative to the high-temperature conformation. Figures in parentheses are changes in chemical shift in ppm.

peratures when the high-temperature form is both a duplex and the predominant species, its tumbling rate is such that NOEs are not observed, even after irradiation times of 1 s.

Protons whose chemical shifts are significantly different between the two forms are listed in Table II. Each of the two spectra represents an average of the spectra of any species in fast exchange, but the two sets of species interconvert by a slow equilibrium. The two forms are effectively duplexes; the imino protons held in the base pairs exchange slowly with bulk water and so can be observed. A saturation transfer experiment was performed to determine whether interconversion of these two duplexes involved a single-stranded intermediate, or some other mechanism involving disruption of base-pair hydrogen bonds. For direct interconversion, it should be possible to observe transfer of saturation between corresponding imino protons in the two forms. On the other hand, mechanisms involving a single-stranded intermediate or some other disruption of the base-pair hydrogen bonds would lead to saturation being lost by transfer to bulk water. This follows since the available evidence (Patel & Hilbers, 1975; Johnson & Redfield, 1981) indicates exchange rates for imino protons with bulk water of $k > 10^5$ s^{-1} in single-stranded oligonucleotides.

When the lower field signal (of the two imino protons) of the low-temperature form (Figure 2) is irradiated for 0.4 s, saturation transfer is seen to the lower field signal of the high-temperature form and vice versa. Irradiation of the higher field signal of the high-temperature form produces an effect to the highest field proton. The reciprocal effect is, however, not observed; this may be due to efficient relaxation of the former resonance. Thus, interconversion of the two forms appears to take place by a mechanism in which the integrity of the base pairs is preserved. This fact tends to rule out a concatenated low-temperature structure in which the 5′ and 3′ halves of each tetramer from base pairs with different oligonucleotide molecules.

Discussion

Structure Derivation. Information provided by NOEs and chemical shifts is sufficient to derive a fairly detailed structure for the low-temperature form. The pattern of NOEs is broadly similar to that observed for the B-DNA oligomers (Reid et al., 1983a), and this standard conformation was taken as the starting point for model building.

NOEs between A_{H-8} and $A_{H-2',H-2''}$ are unusually weak and suggest that these protons are farther apart than in the B-form double helix; alternative explanations necessarily involve a

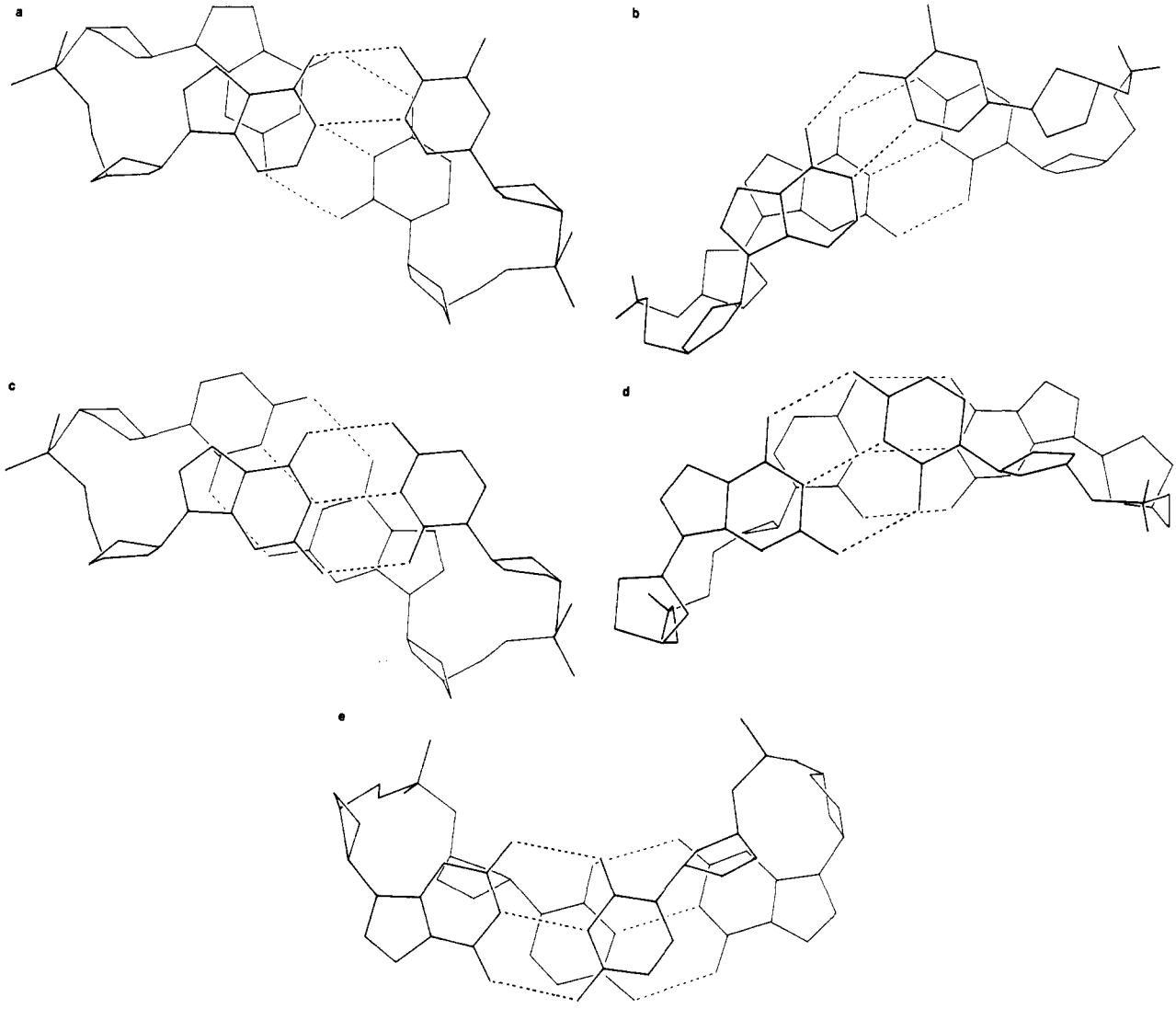

FIGURE 5: Base overlaps. At the T-C step in (a) B-DNA (Arnott & Hukins, 1972) and (b) d(TCGA) low-temperature form. At the C-G step in (c) B-DNA, (d) d(TCGA) low-temperature form, and (e) Z-DNA (Wang et al., 1979).

radically different relaxation pathway and are unlikely. The distance between $A_{H\text{-}8}$ and the 2′-methylene protons of adenosine can be increased both by rotation about the glycosidic bond and by a change in sugar pucker from 2′-endo to 3′-endo. The former effect can produce distances consistent with the NOEs which are observed and provides a more acceptable explanation of the data. As an alternative, however, changing the sugar pucker results additionally in considerable shortening of the $A_{H\text{-}3'}$–A_{H8} distance and would be evident from a conspicuously strong NOE; this is not observed.

When $A_{H\text{-}8}$ is irradiated, the NOE to $G_{H\text{-}1'}$ is considerably greater than that to $A_{H\text{-}1'}$, which is itself of a magnitude usually encountered in B-type oligomers. Enhancement of the NOE must be due to closer approach of $A_{H\text{-}8}$ to $G_{H\text{-}1'}$, than the 3.8A in normal B-DNA. This can be brought about in the model by rotation, largely of the furanose ring, about the glycosidic bond of guanosine. The high anti conformer produced in this way is also consistent with the large NOE seen from $G_{H\text{-}8}$ to $G_{H\text{-}2'}$ and $G_{H\text{-}2''}$.

When NOEs involving guanosine and cytidine are considered, it is clear that some translation of one C·G base pair relative to the other has taken place so as to increase the $G_{H\text{-}8}$ to $C_{H\text{-}1'}$ distance. The remarkably high-field chemical shift of $C_{H\text{-}2'}$ is explained if this shearing brings the C-2′ methylene protons into the shielding zone of the pyrimidine ring of guanine. The distances between $G_{H\text{-}8}$ and $C_{H\text{-}2',H\text{-}2''}$ are little affected (these NOEs are of normal intensity) by this change. A close contact between $C_{H\text{-}2'}$ and the guanine ring can be relieved by a slight propeller twist in both G·C pairs (Fratini et al., 1982).

The extreme weakness of NOEs between $C_{H\text{-}6}$ and the thymidine 2′-protons is best rationalized by using a high anti conformation for T; it does not significantly alter the $C_{H\text{-}6}$–$T_{H\text{-}1'}$ separation compared to B-DNA.

These changes in the geometry of individual nucleotide residues, their orientations relative to one another deduced from the NOE data, and the very distinctive chemical shift of a single proton have some direct consequence for base-pair overlap. The very weak overlap of cytosine and thymine in regular B-DNA (Figure 5a) is considerably improved with only minor change in the relative orientations of guanine and adenine (Figure 5b). The extent of overlap at the C–G junction, poor in B-DNA (Figure 5c), is considerably altered. The result (Figure 5d), however, is strongly reminiscent of the interstrand stacking of cytosine seen in crystals of Z-DNA (Figure 5e) (Wang et al., 1979).

Unfortunately NOEs in the high-temperature form of d(TCGA) could not be detected and rigorous conclusions as to its structure are not warranted. Differences in chemical shift between resonances of corresponding protons in the low- and high-temperature forms are similar to those expected if the latter closely resembles B-DNA. Chemical shift changes

observed on melting of the duplex also support this conclusion. The change in chemical shift of the $C_{H-2'}$ signal based on ring current shielding effects of the different bases (Giessner-Prettre et al., 1976) has already been discussed. The conformational transition brought about by decreasing temperature would move C_{H-6} into the shielding zone of guanine below it. On the other hand, G_{H-8} is moved away from the shielding region of cytosine, and the resonance is deshielded.

Stabilization in the Low-Temperature Form. Different forms of an oligonucleotide duplex in slow exchange on the NMR time scale, although rare, are not unprecedented. Two forms of d(5-MeGC)$_3$ are in slow exchange (Tranh-Dinh et al., 1983) and were taken to represent B and Z conformations. Slow equilibration in this case is not difficult to rationalize in view of the gross structural changes necessary for interconversion. Differences between the two forms of d(TCGA) are, however, more subtle and obstacles to the transformation in terms of high activation energy less apparent.

An important factor in stabilization of the low-temperature form may be the improved stacking of thymine on cytosine. Overall stability of the duplex can be increased by enhancement of the two stacking interactions between the T·A and C·G pairs even at the expense of the single C·G,G·C overlap which is very weak in B-DNA, although it may actually be improved in this reorganization. Similar reasoning has been used to explain the dinucleotide repeating unit in the "alternating" B-DNA conformation of poly[d(A-T)] (Klug et al., 1979). Overlap at the A-T junction is increased at the expense of that at the T-A step since the latter is so poor in regular B-DNA that its deterioration detracts little from the overall stabilization and is more than offset by the overlap gained at the adjacent stacks. In poly[d(A-T)], model-building studies have shown that this optimization of stacking can be accommodated by adjustment in sugar pucker, but in the present cases the rearrangement is more complex.

Hydration is an important contributor to the stability of B-DNA (Drew & Dickerson, 1981) and is likely to be involved in stabilization of the low-temperature form of d(TCGA), but this can only be a matter for speculation.

The inherent stability of both d(TCGA) double helices is surprising. The results reported here were obtained at very low ionic strength and in the absence of metal cations. Melting in neither form of the duplex is apparent below 12 °C, the higher temperature form having $T_m \simeq 25$ °C; increasing salt and nucleotide concentrations tend to favor the low-temperature form. The introduction of sodium ions considerably increases the temperature dependence of chemical shifts, implying additional contributions from fast-exchange equilibria. In 0.1 M NaCl, aggregation is apparent at around 2 °C as NMR lines broaden drastically and the solution is transformed reversibly to a gel.

We initially explored the possibility that the low-temperature form represented some multistranded aggregate analogous to fragments of the d[p(AT)$_2$] crystal lattice (Viswamitra et al., 1978). Interconversion of high- and low-temperature forms, however, occurs without disruption of the Watson–Crick hydrogen bonds and seems to preclude this alternative. Furthermore, we could not succeed in building any plausible oligomeric system consistent with the NOE data.

After it was proposed that improved base stacking is responsible for stabilization of the low-temperature form, the other self-complementary tetramers with similar arrangements of purine and pyrimidine bases were investigated. Both d(CCGG) and d(CTAG) proved unremarkable in terms of chemical shifts and NOE patterns over a range of temperatures and salt concentrations. It appears that the unusual behavior of d(TCGA) is a peculiarity of this sequence.

It is interesting to note the suggested involvement of the pentamer d(TTCGA) in nucleosome organization near the promoter of structural genes for enkaryotic tRNA (Wittig & Wittig, 1982). Nucleosome reconstitution experiments seem to indicate selective binding to this sequence in restriction fragments by H3 dimer in the histone core octamer [J. D. McGhee and G. Felsenfeld, unpublished results; T. J. Richmond, unpublished results [cited in Richmond et al. (1983)]]. It is, however, pure speculation that interaction depends on a conformational predispostion of the nucleotide of the type reported here. The self-complementary hexamer d(TTCGAA) derived from this sequence, which exhibits no slow-exchange behavior, is at present under investigation.

Conclusion

Dependence of the DNA structure on nucleotide sequence may underlie specific or selective interaction with proteins and drugs and therefore provide a key to understanding these phenomena at the molecular level. Certain polymers of defined sequence have distinct conformational preferences (Leslie et al., 1980), while single crystal X-ray studies show appreciable variations in helical parameters from one base pair to the next, particularly in B-DNA (Dickerson & Drew, 1981). High-resolution structural data can at present only be obtained by using model systems based on oligonucleotides, but it is important to interpret the information cautiously. Some of the various forms in which polymeric DNA has been shown to exist appear to be relatively similar energetically and can be interconverted by changes in solution conditions or, in the case of fibers, hydration and counterion type. It is not unreasonable to expect that, with short fragments of double helix, end effects and packing interactions in the crystal can considerably perturb these conformational equilibria. When, as in this report, an oligomer is shown to exhibit a particular sequence-related conformation, it establishes a precedent for analogous situations in polymeric DNA. It may be induced only under torsional stress or other environmental influences such as when bound to a protein but is no less important for this.

This paper describes the use of NMR to characterize an unusual conformation adopted by d(TCGA) in detail, and with a confidence in many respects comparable to X-ray techniques. This duplex structure exists in slow exchange with one which resembles B-DNA. The high activation energy for the interconversion implicit in the observed exchange rate is unexplained, but overall stabilization of the new form which is favored at low temperatures is rationalized on the basis of improved base stacking. The peculiar behavior, however, does not extend to d(CCGG) and d(CTAG) which, with similar sequences of purine and pyrimidine bases, have similar stacking patterns. It is not yet clear whether the structure observed is simply a property of this particular tetramer or whether it persists in the presence of the ordering influence of longer flanking sequences.

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