Processes of Base-Pair Opening and Proton Exchange in Z-DNA†

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ABSTRACT: Using proton magnetic resonance, we have investigated imino and amino proton exchange in the Z form of the four oligomers d(Cbr^{8}GCGCbr^{8}G), d(CGm^{5}CGCG), $d(CG)_6$, and $d(CG)_{12}$. In the latter two oligomers, all five exchangeable protons have been assigned. We find that proton acceptors such as NH_3 or the basic form of Tris enhance imino proton exchange. The base-pair lifetime can then be obtained by extrapolation of the exchange time to infinite concentration of proton acceptor. For $d(CG)_6$ and $d(CG)_{12}$, the values are ca. 3.5 ms at 80 °C and ca. 130 ms at 35 °C. The latter value is about 65 times longer than in the same oligomers in the B form. The activation energy of base-pair opening, 80 kJ/mol, is the same in the Z and the B forms of $d(CG)_{12}$. At 5 °C, the base-pair lifetime is about 3 s, much smaller than the time constant of the Z to B transition, to which it is therefore unrelated. The base-pair dissociation constant at 35 °C, 0.5×10^{-6}, is 5 times smaller than for the same oligomers in the B form. In the absence of added catalyst, at pH 7, the exchange time of the imino proton is 30 min at 5 °C. That of both cytidine amino protons, assigned by NOE, is about 50 min. The longest proton exchange time, ca. 330 min, is assigned unambiguously to the guanosine amino protons. Thus assigned and interpreted in terms of exchange chemistry rather than structural kinetics, the exchange times do not support earlier models of Z-DNA internal motions.

Structural fluctuations of double-stranded nucleic acids leading to solvent exposure of internal groups can be investigated by the exchange of imino protons. In the presence of sufficiently high concentrations of a proton acceptor, acting as a catalyst, imino protons exchange at each opening event, and the imino proton exchange time is equal to the lifetime (τ_0) of the closed base pair. At lower catalyst concentrations the chemical step of exchange from the exposed imino group becomes rate limiting. The imino proton exchange time is then unrelated to the base-pair lifetime (Leroy et al., 1988a,b).

It has been shown that in B-DNA the base pairs open one at a time, their lifetimes are in the range of milliseconds, and they are weakly dependent on the DNA sequence. Deviation from the B form can lead to large changes in the base-pair lifetimes, as recently observed for A·T base pairs of B′-DNA whose lifetimes are up to 100 times longer than those of A·T pairs in B-DNA. This feature was used to study the conditions for formation of the B′-DNA structure and its relation to DNA curvature (Leroy et al., 1988b).

Since the discovery of the Z form of DNA (Pohl & Jovin, 1972) and its crystallographic characterization (Wang et al., 1979), several studies of proton exchange have been performed. By use of tritium–proton exchange in Sephadex columns, two classes of exchanging protons in the Z form of poly[d(GC)] were found (Ramstein & Leng, 1980; Ramstein et al., 1985). By indirect considerations, the three protons in the fast class (exchange half-time 20 min at 0 °C) were assigned to guanosine imino and amino protons and the two in the slow class (exchange half-time 420 min at 0 °C) to cytidine amino protons. It was therefore proposed that there are two base-pair opening modes in Z-DNA, one exposing the guanosine imino and amino groups, and the other exposing the cytidine amino group. These assignments were supported by the observation (Laigle et al., 1989) that H_2O to D_2O solvent substitution causes spectral changes on the Raman lines assigned to guanosine (half-life 13 min at 2 °C) and cytidine (half-life 51 min). Different assignments of the exchange times were obtained in a proton NMR study, which showed that the internal, hydrogen-bonded amino proton of cytidine and the imino proton of guanosine in a 60 base-pair poly[d(GC)] exchange at the same rate (Mirau & Kearns, 1985). This rate was different in 4 M NaCl and in 3.5 M $NaClO_4$ (respectively ca. 100 and 22 min at 4.7 °C), and it was proposed that exchange occurs during transformation of the Z duplex into a conformation intermediate between B and Z. In all of these studies, the effect of catalysts on imino proton exchange kinetics was not observed, and no distinction was made between imino proton exchange and base-pair lifetime.

We now show that the exchange of imino protons of Z-DNA is accelerated by proton acceptors. Base-pair lifetimes, obtained by extrapolation of the exchange times to infinite concentration of catalyst, are orders of magnitude shorter than the values given above. They range from milliseconds at 80 °C to 130 ms at 35 °C. At 5 °C the lifetime is in the range of 3 s. These values will be compared with those of the same molecules in the B form.

Using the nuclear Overhauser effect (NOE), we have assigned the resonances of cytidine and guanosine amino protons. We have measured their exchange times by real-time solvent exchange NMR. At 5 °C and pH 7, the guanosine imino and cytidine amino protons exchange respectively in 30 and 50 min, whereas the guanosine amino protons exchange in 330 min. These exchange times in the absence of added catalyst are in fair agreement with those measured by tritium–proton exchange (Ramstein & Leng, 1980; Ramstein et al., 1985).

For imino and amino protons, exchange is acid catalyzed when the pH is ≤7. Recent observations indicate that this is a property of G·C pairs in both B- and Z-DNA (Guéron et al., 1989).

Experimental Procedures

Oligonucleotide Synthesis. The $d(CG)_{12}$ and $d(CG)_6$ oligonucleotides were synthesized by the β-cyanoethyl phosphoramidite method using a solid-phase Pharmacia gene assembler. Protecting groups were removed by incubation in

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25% ammonia at 50 °C for 48 h. Final purification was achieved by high-pressure liquid chromatography on a PVDI 300-10 column (Société Française de Chromatographie). The d(Cbr^{8}GCGCbr^{8}G) and d(CGm^{5}CGCG) oligomers were kindly supplied by J. M. Neumann (CEN-Saclay) for whom they were synthesized by J. Igolen (Unité de Chimie Organique, Institut Pasteur).

Sample Preparation. The oligonucleotides were passed through a Chelex-100 column to remove paramagnetic impurities and lyophilized. NMR samples were prepared by dissolving 50 OD of the duplex in 0.2 mL of 90% H_2O–10% D_2O.

The salt concentration was chosen so that the duplexes would be in the Z conformation. For the d(CG) oligomers, the salt was either NaCl (4 M) or $NaClO_4$ (3.5 M). The NMR spectra were the same in both salt conditions, except for larger line widths at low temperature in NaCl, suggesting that aggregation (Genest et al., 1987) is more pronounced in NaCl than in $NaClO_4$. The experiments on the B form of $d(CG)_{12}$ were carried out at 0.1 M NaCl concentration.

The d(Cbr^{8}GCGCbr^{8}G) hexamer, which adopts the Z conformation even in low salt (Taboury et al., 1985), was studied in 0.1 M NaCl. The d(CGm^{5}CGCG) duplex was studied in 4 M NaCl.

For exchange measurements, ammonia was added to the sample in small portions (1–10 μL) from concentrated stock solution. The pH, measured at room temperature, was 8.8 for experiments performed with ammonia and 7.5 when the catalyst was Tris. It was measured after each addition of catalyst and adjusted, if necessary, with HCl or NaOH (0.1 M).

NMR Methods. The experiments were performed either at 276 MHz or, for the data of Figures 1, 6, and 7, at 360 MHz. The JR sequence (Plateau & Guéron, 1982) was used for solvent signal suppression. Selective irradiation was achieved with a hard-pulse DANTE sequence (Morris & Freeman, 1978) as previously described (Leroy et al., 1988a). For T_1 or magnetization transfer measurements, the magnetization was inverted. For amino proton assignments by NOE, the magnetization was rotated continuously by the DANTE sequence, so that its average longitudinal value was zero. Proton spectra were calibrated with respect to an internal reference of 4,4-dimethyl-4-silapentane-1-sulfonate (DSS).

Counting of the protons in a resolved line was performed by computing the area under the curve. In the case of imperfectly resolved lines, deconvolution was done by hand, and integration by paper weighing. The result is designated in the text as the intensity of the line. For comparison of the intensities of two lines, the JR sequence was set up to provide equal sensitivities at their two frequencies.

Determination of Exchange Times. The imino proton exchange times were obtained from real-time proton–deuterium exchange experiments at low temperature or from the rates of longitudinal relaxation or of magnetization transfer from water at higher temperatures.

(*A*) *Proton–Deuterium Exchange.* The dry sample (10–100 OD) was dissolved in 25 μL of a H_2O solution at the appropriate salt concentration (see sample preparation). The liquid was drawn into a 3 ft long Teflon capillary (1-mm i.d.) connected to a syringe, and pulled up so as to generate a 10 mm long air gap at the tip of the capillary. The capillary was inserted into an NMR tube containing 220 μL of D_2O in the same ionic conditions, so that the tip touched the D_2O surface. The NMR tube was placed into the temperature-regulated probe. After waiting for thermal equilibrium, the oligomer–H_2O solution was quickly injected in the D_2O solution. Mixing was accelerated by the injection of air bubbles. Data acquisition could start 20 s later, allowing the determination of exchange times as short as 1 min.

The results reported here were obtained with 10 OD in 25 μL of H_2O as the starting solution. Under these conditions, exchange of the imino protons is exponential. We avoided higher concentrations because of the observation that they cause part of the imino protons (up to half when starting from 100 OD in 25 μL) to exchange in the first seconds after injection, whereas the remainder exchange at the same rate as that in more dilute conditions.

(*B*) *Proton–Proton Exchange.* Exchange times shorter than 1 s were measured by relaxation or by magnetization transfer (Leroy et al., 1988a). The imino proton relaxation rate in the presence of catalyst is related to the proton exchange rate increment τ_{excat}^{-1} by

$$\tau_{excat}^{-1} = T_{1cat}^{-1} - T_{10}^{-1} \quad (1)$$

where T_{1cat} and T_{10} are the longitudinal relaxation times with and without added catalyst.

The exchange time τ_{ex} is given by

$$1/\tau_{ex} = 1/\tau_{excat} + 1/\tau_{AAC} \quad (2)$$

where τ_{AAC} is the exchange time in the absence of added catalyst, which is obtained from the variation of imino proton magnetization versus time after selective inversion of the water protons.

Theory of Exchange

Imino proton exchange from a base pair is a two-step process involving opening of the base pair followed by chemical exchange from the open state (Teitelbaum & Englander, 1975). This leads to

$$\tau_{excat} = \tau_0 + \tau_i/\alpha K \quad (3)$$

where τ_{excat}^{-1} and τ_i^{-1} are the imino proton exchange rates for a duplex and for a mononucleotide at the same catalyst concentration. The time τ_0 is the base-pair lifetime, and K is the base-pair dissociation constant. The factor α accounts for differences in catalysis from the open base pair and from the nucleoside, such as for instance hindered access for a bulky or negatively charged catalyst (Guéron et al., 1989).

In eq 3, the effect of the catalyst concentration issues from τ_i. Exchange in the nucleoside is a function of this concentration [C], of the pKs of the nucleoside and of the catalyst pK_n and pK_c, and of the collision rate k_{coll} (Eigen, 1964):

$$\tau_i^{-1} = k_{coll}[C]/(1 + 10^{pK_n - pK_c}) \quad (4)$$

A plot of the imino proton exchange parameter τ_{excat} as a function of the inverse of the catalyst concentration is therefore a straight line whose extrapolation to infinite concentration of catalyst yields the closed-state lifetime.

Results

Assignment of All Exchangeable Protons of Internal Base Pairs in the $d(CG)_{12}$ and $d(CG)_6$ Oligomers. The imino proton spectrum of these duplexes is a single line at 13.05 ppm. The assignment of the amino protons is complicated by line overlap and by the temperature-dependent rotation of the G amino group. To achieve the complete assignment, we have used both NOEs (Figure 2) and exchange kinetics (Figure 6).

At 65 °C, the assignment of the amino protons (given in Figure 1) is relatively straightforward. First, exchangeable

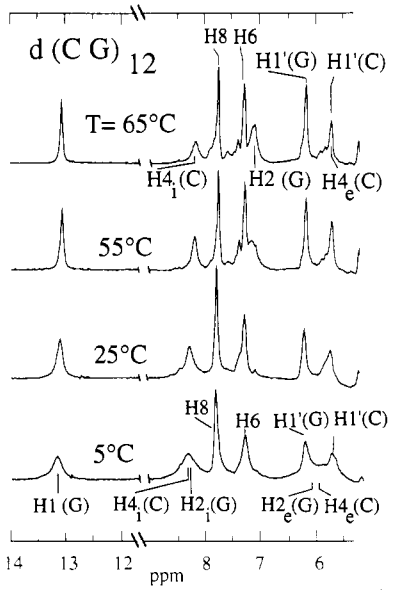

FIGURE 1: NMR spectra of the $d(CG)_{12}$ duplex at different temperatures. The assignments of the nonexchangeable protons are from Patel et al. (1982); those of the amino protons $H_4(C)$ and $H_2(G)$ are obtained in this work. The resonances of the internal and external G amino protons are distinct at low temperature but merge at high temperature. Experimental conditions: 3.5 M $NaClO_4$, 10% D_2O, pH 7.3; proton frequency, 360 MHz. A line-narrowing procedure has been used.

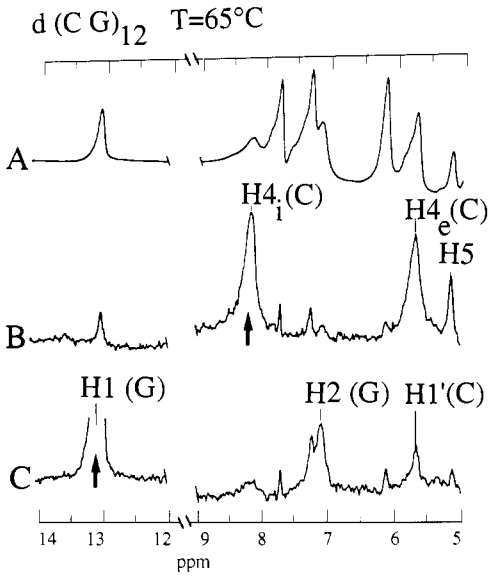

FIGURE 2: Assignments of the exchangeable protons of the $d(CG)_{12}$ duplex at 65 °C. Reference spectrum A and NOE difference spectra obtained after irradiation (arrow) of the resonance at 8.3 ppm (B) or of the imino proton resonance at 13.05 ppm (C). The NOE to the cytidine amino protons in spectrum C is weak due to the fast exchange of these protons. The NOE at 5.7 ppm occurs because of the short distance between the guanosine imino proton and the H1′ of the neighboring cytidine on the same strand in the 3′ direction. The small-intensity peaks are due to spin diffusion during the 150-ms irradiation period. Experimental conditions: 3.5 M $NaClO_4$, pH 8. No line narrowing.

lines were located at 8.2, 7.1, and 5.7 ppm on the difference spectrum of spectra in H_2O and in D_2O (not shown). Second, these lines were studied by NOE (Figure 2).

The 8.2 and 5.7 ppm lines are NOE interconnected and are also NOE connected to the imino proton line and to a line at 5.2 ppm, previously assigned (Patel et al., 1982) to the H5 proton of cytidine. Hence, they are assigned to the internal and external amino protons of C, respectively. Their intensities, estimated from the difference spectrum (or directly from the spectrum, as regards the 8.2 ppm line) are equal to 1, with the imino line as reference.

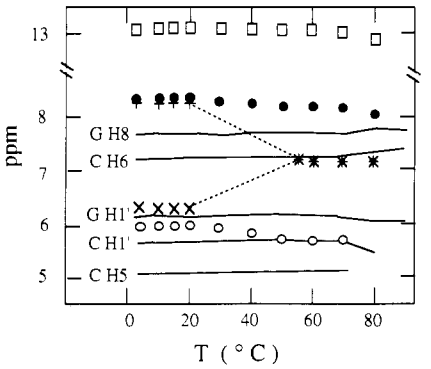

FIGURE 3: Chemical shifts, referenced to DSS, of the exchangeable and aromatic protons of the $d(CG)_6$ and $d(CG)_{12}$ duplexes as a function of temperature: (□) G imino proton; (●, ○) internal and external C amino protons; (+, ×) internal and external G amino protons ($T \leq 20$ °C); (*) G amino protons in fast rotational exchange ($T \geq 45$ °C). Experimental conditions: 3.5 M $NaClO_4$, 10% D_2O.

The remaining exchangeable peak, at 7.1 ppm, is assigned to the two G amino protons. It is NOE connected to the imino proton (Figure 2C), and its intensity is indeed 2. In B-DNA, the G amino protons also give rise to a single resonance, and this is explained by rotation of the amino group (Patel, 1977; McConnell, 1984).

At lower temperatures, the NOE assignment of the C amino protons can be made in the same way as that at 65 °C, and the change in chemical shift with temperature is small (Figure 3).

By contrast, the 7.1 ppm line assigned to G amino protons broadens and disappears below 45 °C (Figure 1). At still lower temperatures, the G amino protons give rise to two resonance lines at nearly the same chemical shifts as the C amino protons. This is shown, first, by an increase in the intensity of the downfield amino proton line (8.2 ppm at 65 °C; 8.3 ppm at 5 °C), from 1 ± 0.2 at 25 °C to 1.7 ± 0.3 at 5 °C, the reference intensity being that of the imino proton line (Figure 1). The extra intensity is exchangeable and is therefore assigned to one of the G amino protons.

The second piece of evidence is the direct separation by differential exchange of the spectra of the C and G amino protons (Figure 6), as discussed below.

In agreement with these assignments, the middle point of the position of the G amino proton resonances at 15 °C is 7.0 ppm (Figure 3), to be compared with the single position, 7.1 ppm, at 55 °C.

Catalyst-Dependent Exchange. (A) Base-Pair Lifetime. At high temperatures, base-pair lifetimes were obtained by extrapolation of the imino proton exchange time to infinite catalyst concentration (Figure 4A). Similar values were found for $d(CG)_6$ and $d(CG)_{12}$. They range from 3.5 ms at 80 °C to 130 ms at 35 °C (Figure 5A).

Imino proton exchange times at 5 °C were measured by direct exchange in D_2O solutions. Ammonia (pH 8.8, 70 mM) reduces the exchange time of the imino proton of $d(GC)_{12}$ from 30 min to less than the lower experimental limit of 1 min. Hence, the base-pair lifetime at 5 °C is certainly less than 1 min. By extrapolation of the lifetimes measured above 35 °C, an estimate of 3 s is obtained (Figure 5A).

The base-pair lifetimes of $d(CG)_{12}$ in the B-form were also determined (Figure 4B). We find that the activation energy for opening a G·C base pair is the same, 80 kJ/mol, in the Z and B forms. The base-pair lifetime is 65 times longer in the Z than in the B form.

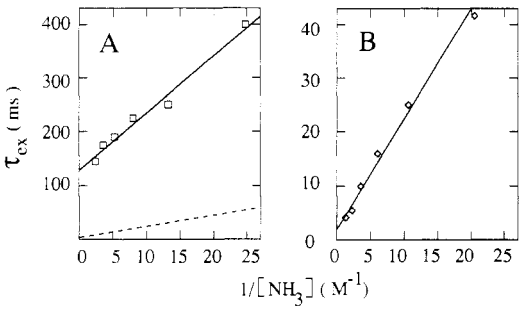

FIGURE 4: Exchange times of the guanosine imino protons of $d(CG)_{12}$ in the Z form (panel A, 4 M $NaClO_4$) and in the B form (panel B, 0.1 M NaCl; also represented by the dotted line in panel A). The exchange time varies linearly with the inverse of the ammonia concentration. The base-pair lifetimes, obtained by extrapolation to infinite concentration, are 130 and 2 ms, respectively. The exchange times are measured by magnetization transfer from water (Z-DNA) or derived from the effect of ammonia on longitudinal relaxation (B-DNA). Experimental conditions: 0.1 M NaCl, 10% D_2O, pH 8.8, T = 35 °C.

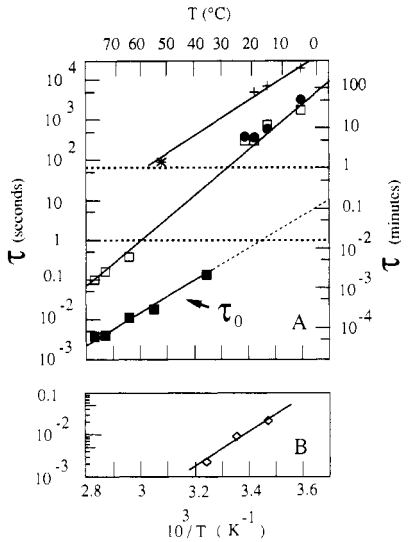

FIGURE 5: (A) Base-pair lifetime (■) and proton exchange times in the absence of added catalyst are plotted versus 1/temperature in the case of $d(CG)_{12}$ in the Z form: (□) imino proton; (●) internal cytidine amino proton; (+) internal guanosine amino proton; (*) guanosine amino protons in fast rotational exchange. For both C and G, the exchange time of the external amino proton (not shown) is close to that of the internal one. However, its measurement is imprecise due to the proximity of the water resonance. Exchange times between 1 and 60 s (horizontal dotted lines) are inaccessible to measurement. (B) Base-pair lifetime of the $d(CG)_{12}$ duplex in the B form ([NaCl] = 0.1 M).

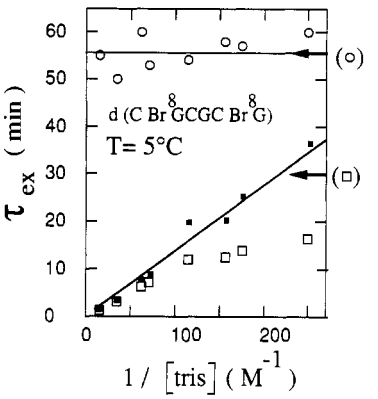

FIGURE 6: Effect of Tris on exchange of guanosine imino (□, ■) and cytidine amino protons (○) of the central base pair of d-(CBr^{8}GCGCBr^{8}G). The amino proton exchange times are independent of the Tris concentration. Imino proton exchange is strongly affected, and the extrapolation to infinite concentration gives an upper limit of 1 min for the base-pair lifetime. The arrow indicates in each case the exchange time in the absence of Tris. The full squares are computed as the inverse of the exchange rate increment upon addition of Tris.

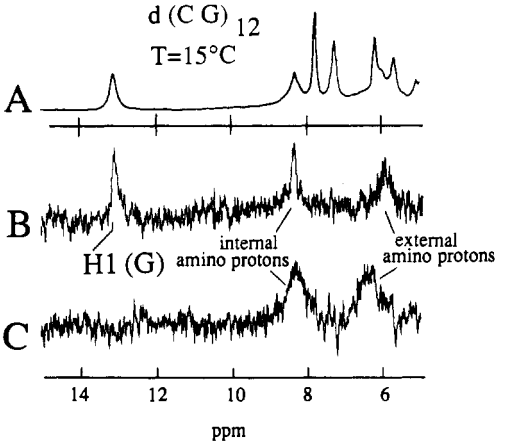

FIGURE 7: Real-time solvent exchange experiments on the $d(CG)_{12}$ duplex at 15 °C, pH 7. Spectrum A is a reference spectrum of the oligomer in 90% H_2O/10% D_2O and 3.5 M $NaClO_4$. Spectrum B is obtained by subtracting the spectrum recorded at t_2 = 20 min from that recorded at t_1 = 3.5 min. It contains lines from exchangeable protons only and strongly emphasizes signals from protons that exchange in the range of 3 min: the G imino proton and the internal (narrow) and external (broad) amino protons of cytidine. Spectrum C is obtained similarly, but with t_1 = 115 min and t_2 = 800 min; the signal is amplified 20 times over that of spectrum B. The two amino protons of guanosine are conspicuous. The internal amino proton has the same chemical shift as that of cytidine. Proton frequency, 360 MHz.

(B) Base-Pair Dissociation Constant. The dissociation constant is estimated as the ratio of the imino proton exchange time in isolated guanosine and in the duplex, the latter value being measured under conditions where the exchange time is much longer than the base-pair lifetime, and is controlled by the added catalyst (Guéron et al., 1989). For the exchange time of the imino proton of the guanosine monomer at 35 °C, we measure τ_{ex} (s) = $(5.6 \times 10^{-9})[NH_3]$. With the data of Figure 4A, this gives $K = 0.5 \times 10^{-6}$ at 35 °C, and 5 times more for the dissociation constant of the B form (Figure 4B). The base-pair lifetimes, 130 and 2 ms respectively, can also be read off the figure. The open-state lifetime (equal to the product of the base-pair lifetime and the dissociation constant) is therefore longer in the Z form (65 ns) than in the B form (5 ns) of this duplex.

Exchange in the Absence of Added Catalyst. (A) $d(CG)_{12}$ and $d(CG)_6$ Duplexes. Imino and amino proton exchange times at neutral pH and in the absence of added catalyst were measured by real-time solvent exchange experiments between 5 and 52 °C. The imino proton exchange time was also measured at high temperature (≥65 °C) by magnetization transfer from water. In all cases the G amino protons have the slowest exchange rate.

Figure 7 presents data from a real-time solvent exchange experiment performed at 15 °C, pH 7, on $d(CG)_{12}$. Spectrum B is the difference between spectra recorded 3.5 and 20 min after dilution of the oligonucleotide in D_2O: it shows the faster exchanging protons. Spectrum C, the difference between spectra obtained 115 and 80 min after dilution, shows the slower exchanging ones.

These spectra demonstrate directly that the 8.3 ppm peak indeed includes two lines at 15 °C, whose widths and exchange times are quite different. At 5 °C, the two components that are assigned to the C and G internal amino protons have

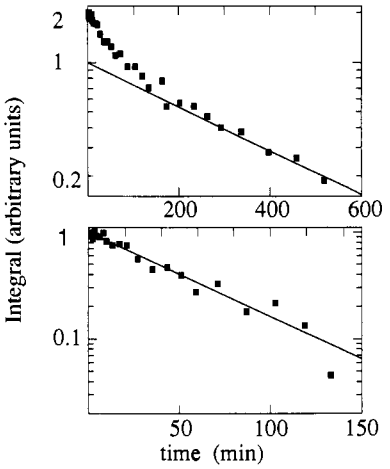

FIGURE 8: Intensity of the 8.3 ppm line of the $d(GC)_{12}$ duplex as a function of time after solvent exchange at 5 °C. The intensity in the first spectrum, recorded 45 s after dilution in D_2O, is assigned a value of 2 arbitrary units. The slow phase is fitted to a single expontential (solid line): the intensity is 1, and the time constant is 330 min (top). The residual intensity also decays exponentially: the intensity is the same, and the time constant is 55 min (bottom).

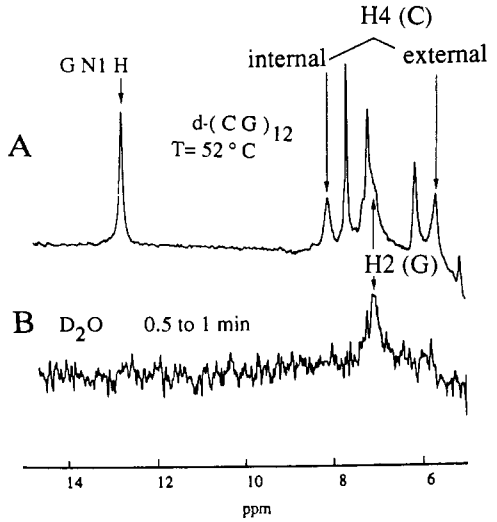

FIGURE 9: Real-time solvent exchange experiment at 52 °C. (A) Reference spectrum; (B) difference between a spectrum recorded in 0.5 min, starting 0.5 min after dilution, and a spectrum recorded after complete exchange of all exchangeable protons (20 min). See also Figure 7. The G amino protons are the slowest to exchange.

comparable intensities (Figure 8).

At 22 °C, by contrast, the decay (not shown) is exponential (τ = 5 min), and the total intensity of 1 corresponds to a single proton. The 8.3 ppm line is assigned to the cytidine internal amino proton by continuity with the high-temperature spectra, as discussed under Assignment of All Exchangeable Protons of Internal Base Pairs in the $d(CG)_{12}$ and $d(CG)_6$ Oligomers (Figure 3).

Exchange times were determined between 5 and 25 °C from spectra taken at different times after dilution in D_2O, as in Figures 7 and 8. An example of exchange processes at higher temperatures is shown in Figure 9. In the spectrum taken between 0.5 and 1 min after dilution in D_2O at 52 °C, the G amino protons, assigned at 7.1 ppm (Figure 2), are still visible, whereas the C amino and G imino protons have already disappeared. The exchange times are 90 s for the former and less than 20 s for the latter. On the basis of extrapolations from the lower temperature values (Figure 5A), the slower exchanging protons at 8.3 and 6.3 ppm ($T \leq 20$ °C) are assigned to the G amino protons.

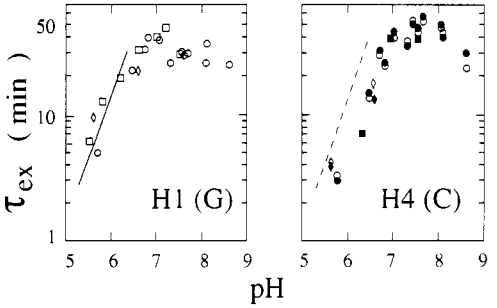

FIGURE 10: Exchange times versus pH at 5 °C, for $d(CG)_6$ (♦, ◇), $d(CG)_{12}$ (■, □), and $d(Cbr^8GCGCbr^8G)$ (○, ●). (Left) Guanosine imino proton. At low pH, the exchange time varies as $1/[H^+]$ (solid line). (Right) Cytidine amino protons. The filled and open symbols stand respectively for internal and external amino protons. The exchange times also vary as $1/[H^+]$. They are even shorter than the exchange time of the imino proton (dotted line).

(B) Other Duplexes. We have also measured the exchange of imino and amino protons of the $d(Cbr^8GCGCbr^8G)$ and $d(CGm^5CGCG)$ hexamers. For these duplexes, the imino proton lines of the different pairs are partly resolved (Taboury et al., 1985). In the absence of added catalyst and at pH 7, the exchange times of imino and cytidine amino protons of the central base pair are comparable to those of $d(CG)_6$ and $d(CG)_{12}$. Those of the first and second base pairs are at least 1 order of magnitude smaller. The G amino protons are not observed at low temperature. Figure 6 shows that Tris base catalyzes the exchange of the G imino proton but has no effect on the C amino protons, as expected from their high p*K* (McConnell, 1984).

Acid-Catalyzed Proton Exchange. Proton exchange in Z-DNA is acid-catalyzed. Figure 10 summarizes the exchange behavior of G imino and C amino protons vs pH, at 5 °C. Exchange times are independent of pH between pH 7 and pH 8 and decrease linearly as the inverse of $[H^+]$ for pH ≤6.5. The pH-dependent exchange process is even more efficient for the C amino protons than for the G imino proton. The G amino proton exchange time is 330 min at pH 7 and 160 min at pH 6. A similar pH effect has also been observed in B-DNA (Guéron et al., 1989).

Discussion

Base-Pair Lifetime. The variation of the imino proton exchange time upon addition of exchange catalyst clearly indicates, as for B-DNA, the existence of an exchange pathway in which the chemical step of exchange is limiting in usual buffer conditions. The base-pair lifetime, obtained by extrapolation of the exchange time to infinite catalyst concentration (Figure 4A), is shown in Figure 5 for different temperatures. It is about 3 ms at 80 °C and 130 ms at 35 °C. Extrapolation from higher temperatures gives values of 1 s at 15 °C and 3 s at 5 °C. The measurements give an upper limit of 1 min for the base-pair lifetime at 5 °C.

Base-pair lifetimes are now available for three DNA structures. At 15 °C, the lifetimes in B-DNA are in the range of 20 ms for G·C and a few milliseconds for A·T pairs (Leroy et al., 1988a). In the DNA-curving structure (B′-DNA) observed for sequences containing stretches of A, the lifetime of A·T base pairs in the stretch may be longer than 100 ms (Leroy et al., 1988b). The G·C base-pair lifetime in Z-DNA is even larger, ca. 1 s.

The base-pair lifetime is the same for $(GC)_{12}$ and $(GC)_{24}$, and it is weakly dependent on salt concentration. This is in contrast to the time constant of the Z to B transition, which is strongly dependent on oligomer length and salt concentration. Furthermore, the base-pair lifetime (0.4 s at 25 °C in

4 M NaCl; see Figure 5) is thousands of times shorter than the time constant of the Z to B transition under similar conditions (Pohl & Jovin, 1972). Hence, imino proton exchange in Z-DNA is unrelated to this transition.

In B- and B′-DNA, we showed that neighboring base pairs have different lifetimes and concluded therefrom that base pairs open one by one. In Z-DNA, even though the structural repeat is made of two base pairs, symmetry implies that base pairs are identical in all their properties, be they kinetic (for instance, the opening rate) or chemical (for instance, the chemical shift of the imino proton). It is therefore not possible to determine by the method used for B- or B′-DNA whether the opening process involves only one base pair.

The activation energy for base-pair opening is expected to be larger if base pairs open cooperatively. Previously we measured activation energies in the range of 43–65 kJ/mol for base pairs of B-DNA, which open one by one, by the criterion of different lifetimes (Leroy et al., 1988a). In B′-DNA, a value of 75 kJ/mol was found (Leroy et al., 1988b). In the case of $(dA)_{14}\cdot(dT)_{14}$ for which we expect by analogy (but cannot prove) that opening is one pair at a time, the value was 100 kJ/mol. We now find that for an alternating GC oligomer in both the B and Z forms the activation energy is 80 kJ/mol (Figure 5), comparable to the above values, if on the high side. We doubt that one could argue on this basis for simultaneous opening of more than one base pair.

Acid-Catalyzed Exchange. Acid-catalyzed exchange of G imino protons has already been observed for B-DNA (Guéron et al., 1989). This is however the first observation of acid-catalyzed exchange of G and C amino protons in DNA duplexes.

The analysis of the exchange mechanism is beyond the scope of this paper. For B-DNA it has been proposed (Guéron et al., 1989) that acid-catalyzed exchange might occur during transient formation of Hoogsteen $CH^+\cdot G$ base pairs, and this raises the question of a second exchange process. The Z-DNA structure may be well suited to the study of this problem, due to the possibility of investigating the exchange of all of the exchangeable protons.

Comparison with Related Studies. The *base-pair lifetimes* reported here are much shorter than those derived in previous studies where the effect of added catalysts on imino proton exchange had gone unnoticed (Ramstein & Leng, 1980; Mirau & Kearns, 1985), and which therefore correspond to proton exchange times measured under conditions where the chemical exchange step is rate limiting.

The situation for Z-DNA is comparable to that of B-DNA some years ago. The reasons invoked in the case of B-DNA (Guéron et al., 1987) also explain why catalyst effects have gone unnoticed in Z-DNA. First, imino proton exchange times are finite even in the absence of catalyst, leading to a concentration threshold below which the effect of added catalyst is not apparent. Second, the acceleration of exchange requires large concentrations of catalyst due to the small value of the base-pair dissociation constant.

We do not know why catalysis was not observed in the Raman study (Laigle et al., 1989), using 500 mM Tris, pH 7. This corresponds to a 50 mM concentration of the basic form of Tris, a concentration which reduces the imino proton exchange time of the central base pair of the d-($Cbr^8GCGCbr^8G$) hexamer to ca. 1 min (Figure 6).

There is also a discrepancy concerning the *assignment of exchange times* to the different amino protons in the absence of added catalyst. The comparison of the different studies is complicated by differences in compounds used and in sample preparation and by the sensitivity of proton exchange to pH, even in the neighborhood of pH 7. Nevertheless, we are confident that the much more direct NMR assignments are stronger than those derived from comparative studies of tritium exchange (Ramstein & Leng, 1980; Ramstein et al., 1985; Hartmann et al., 1982). Our finding that the long (330 min) and short (10 min) exchange times both relate to G, rather than to C and G, respectively, and furthermore reflect chemical rather than kinetic events, makes unnecessary the kinetic hypothesis derived earlier to explain them, namely, the existence of different opening processes responsible for exchange of protons from G and C, respectively.

The *values* of the exchange times reported for C amino protons (Laigle et al., 1989; Mirau & Kearns, 1985) are generally comparable to those reported here in the absence of catalyst (Figure 5). However, we did not find any difference between the exchange times measured in 4 M NaCl and 3.5 M $NaClO_4$, contrary to the previous report. The long exchange time (ca. 330 min at 5 °C, pH 7), first observed by tritium exchange (Ramstein & Leng, 1980; Ramstein et al., 1985) and assigned here to G amino protons, was not observed either in the Raman study (Laigle et al., 1989) or in the NMR study (Mirau & Kearns, 1985). In the second case, this is probably because the only amino line studied was the one at 8.3 ppm, which has contributions from amino protons of both C and G.

Conclusions

This study extends to Z-DNA the observations of base-pair opening at temperatures well below melting. As in B- and B′-DNA, disruption of the duplex involves a small number of base pairs, probably one. This process is much slower than that in B- or even B′-DNA, but its dissociation constant is only 6 times less than that for G·C base pairs of B-DNA.

As in B- and B′-DNA, the opening process is unrelated to global changes such as duplex strand dissociation or the Z to B transition. It is only in physical conditions close to those which correspond to these cooperative events that the kinetics of base-pair opening should be affected.

The long exchange times observed in Z-DNA are favorable for the investigation of amino proton exchange. The surprising observations that the external and internal amino protons of cytidine exchange at the same pH-dependent rate and that this rate is comparable to that of the imino proton in the absence of external catalyst are indicative of a novel structural fluctuation which is revealed at low pH but should also exist at pH 7.

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Hydration of dA·dT Polymers: Role of Water in the Thermodynamics of Ethidium and Propidium Intercalation†

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ABSTRACT: We report differences in the interaction of two structurally similar phenanthroline intercalators, ethidium and propidium, with poly(dA)·poly(dT) and poly[d(A-T)] as a function of ionic strength based on titration microcalorimetry, fluorescence titration, and hydrostatic pressure measurements. Both ethidium and propidium bind more strongly to poly[d(A-T)]·poly[d(A-T)] than to poly(dA)·poly(dT). Ethidium intercalation into the latter polymer displays titrations with positive cooperativity; this is not found with propidium. The enthalpy of intercalation ($\Delta H°$) is exothermic for both dyes with poly[d(A-T)]·poly[d(A-T)]; however, the value of this parameter is nearly zero in the case of poly(dA)·poly(dT). The molar volume change ($\Delta V°$) accompanying dye intercalation is negative under all conditions for poly[d(A-T)]·poly[d(A-T)] whereas it is positive for poly(dA)·poly(dT). The changes observed in $\Delta V°$ correlate well with the entropy changes derived from the titration and calorimetric data for this reaction. The results, interpreted in terms of the relative hydration of these two polymers, are consistent with a higher extent of hydration of poly(dA)·poly(dT) relative to poly[d(A-T)]·poly[d(A-T)].

The hydration of helical nucleic acids remains a subject of intensive experimental and theoretical investigation. The particular conformation of double-stranded DNA depends on its primary sequence and its degree of hydration; sequence-dependent hydration has been inferred from X-ray analysis of the crystal structures of oligonucleotides and other physical techniques (Drew & Dickerson, 1981; Kopka et al., 1983; Kennard et al., 1986; Wang et al., 1979; Buckin et al., 1989). Presumably, specific water–DNA interactions arise primarily from hydrogen bonding to the groups on the edges of the bases facing the grooves of the double helix and to a lesser extent from hydrogen bonding to the sugars and phosphate groups. The conformational plasticity of DNA and sequence-dependent ligand binding can be rationalized in terms of the hydration at these positions (Saenger et al., 1986). Experimental techniques that change the water activity shift the equilibrium between the various conformations of DNA and alter the characteristics of the interaction of DNA with ligands (Pohl & Jovin, 1972; Ivanov et al., 1973; Pohl, 1976; Rich et al., 1984).

The partial molar volume of water involved in the hydration of a solute is smaller than that of bulk water; volume changes during association reactions reflect changes in electrostriction of water molecules around charges or changes in the solvent cage surrounding hydrophobic molecules (Drude & Nernst, 1894; Frank & Evans, 1945; Kauzmann, 1959). As a consequence of their size and complexity, both of these effects often contribute significantly to the volume change of reactions and conformational changes involving biological molecules. The volume change accompanying formation of a DNA duplex from single strands is nearly zero because of compensation between electrostriction and hydrophobic effects (Heden et al., 1964; Weida & Gill, 1966; Chapman & Sturtevant, 1966; Noguchi et al., 1971; Gunter & Gunter, 1972; Hawley & MacLeod, 1974, 1977). Determination of the molar reaction

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