Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 1594–1598, March 1985
Biochemistry

Unusual proton exchange properties of Z-form poly[d(G-C)]

(NMR/DNA "breathing"/B → Z kinetics/DNA dynamics)

PETER A. MIRAU* AND DAVID R. KEARNS†

Department of Chemistry, University of California-San Diego, La Jolla, CA 92093

Communicated by Bruno H. Zimm, October 29, 1984

ABSTRACT **Real-time solvent-exchange NMR has been used to study the proton exchange in Z-form poly[d(G-C)]. In 4.5 M NaCl the most slowly exchanging protons (about two orders of magnitude slower than in B-DNA) are identified as the guanine imino proton and the cytosine amino proton hydrogen bonded to the guanine carbonyl. Both protons exchange at the same rate and the exchange follows single-exponential kinetics and cannot be catalyzed, implying that the exchanges of the two protons both occur from the same transient solvent-exposed state. The exchange depends strongly on temperature and the activation energy for exchange (≈22 kcal/mol) is the same as the activation energy for the B → Z transition. The rate of proton exchange is identical with the B → Z transition rate, both measured in 4.5 M NaCl. The correlation between the B → Z kinetics and the proton exchange also extends to 3.25 M $NaClO_4$ solutions, in which both rates are 5 times faster. This unexpected parallelism between the B → Z transition kinetics and the Z exchange kinetics indicates that the rate-limiting steps in the two processes are similar.**

The ability of certain DNA sequences to adopt alternative conformations is one manifestation of the sequence-dependent properties of DNA. The most notable example is the conversion from B-DNA to Z-DNA, observed for poly[d(G-C)] in solution (1) and later for [d(C-G)$_3$·d(C-G)$_3$] and [d(C-G)$_2$·d(C-G)$_2$] in the crystal (2, 3). Since that time, it has been shown that Z-DNA exists in supercoiled plasmids under physiological conditions (4, 5). Antibodies to Z-DNA bind to the DNA from a wide variety of organisms (6–8), and the discovery of Z-DNA-binding proteins (9) suggests that Z-DNA plays some role *in vivo*.

B- and Z-DNA differ not only in conformation but also in their dynamic properties. Leng and co-workers (10, 11) discovered that the rate of exchange of two protons in Z-form poly[d(G-C)] was orders of magnitude slower than in B-form DNA. At 0°C, two protons exchange with a 7-hr half-life, and three protons exchange in 20 min. B-form poly[d(G-C)] at 0°C also exhibits four protons that exchange with a 20-min half-life, with one other proton exhibiting a much faster exchange rate. Since two very slowly exchanging protons were observed in the Z form of poly[d(I-br^{5}C)·d(I-br^{5}C)] (11, 12), Ramstein, Leng, and their colleagues reasoned that the two most slowly exchanging protons in the Z-DNA must be the cytosine amino protons (C_{am}) and analyzed their exchange data accordingly.

We have been studying the exchange of the imino protons in short (~60-base-pair) DNA polymers by NMR relaxation techniques (13–15), but we found that exchange from Z-form poly[d(G-C)] was too slow (<1 s^{-1} at 85°C) to use this approach. Nevertheless, using our data to estimate the exchange rate at high temperature and the Ramstein and Leng (10) rate obtained at low temperature, we calculated an apparent exchange activation energy of only 12 kcal/mol (1 kcal = 4.18 kJ). This value seems unreasonable because the activation energy for exchange from the free base is 12 kcal/mol (16) and the values for double-stranded DNA and RNA polymers are typically 16–22 kcal/mol (13–17). Since exchange in Z-DNA is so slow, an activation energy of at least 22 kcal/mol is expected (14). To examine the slowly exchanging protons in Z-DNA, we have used the real-time solvent-exchange technique previously used to study proton exchange in proteins (18) and tRNA (19). Contrary to the proposed assignments, we find that the most slowly exchanging pair of protons in Z-DNA are not the C_{am}, but rather the guanine imino (G_{im}) and the cytosine amino proton hydrogen bonded to the G carbonyl, and this indicates that the rate of opening of base pairs in Z-DNA is 1/20th as fast (half-life of 7 hr at 0°C) as the very slow rate previously inferred (10). There is a remarkable similarity in the rate constant for the B → Z transition and the exchange rate for the most slowly exchanging proton in Z-DNA in that: (*i*) the activation energies are nearly identical (≈22 kcal/mol), (*ii*) the absolute rate constants are identical, and (*iii*) both rates are 5 times faster in 3.25 M $NaClO_4$ than in 4.5 M NaCl. These results suggest that the rate-limiting steps in the B → Z transition are similar to those leading to exchange from Z-DNA. Studies on Z-form polymers containing both A·T and G·C base pairs show that the same considerations apply to other Z-forming sequences (unpublished results).

MATERIALS AND METHODS

Poly[d(G-C)] (P-L Biochemicals, lot 676-7) was sonicated to ≈60 base pairs (14, 15), precipitated with ethanol, dissolved in 0.125 ml of buffer containing 4.5 M NaCl (or 3.25 M $NaClO_4$) and 0.01 M sodium cacodylate at pH 7, and placed in a Wilmad 508-CP NMR microcell. Real-time solvent-exchange experiments were performed by first equilibrating the Z-DNA in buffer containing ^1H_2O. The sample was placed in the NMR spectrometer and the instrumental parameters were adjusted at the desired temperature. The solvent was removed with a stream of nitrogen and the DNA/salt mixture was dissolved in ^2H_2O at 0°C and returned to the spectrometer, where the exchange of protons for deuterons was measured as a function of time. Even with this solvent-exchange procedure, the residual $^1HO^2H$ peak was large (≈1%), so the spectra were acquired with the "1–1" solvent suppression sequence (20). One consequence of this approach is that the relative intensity of the peaks depends on the choice of experimental parameters, and peaks within ≈2 ppm of the $^1HO^2H$ line are strongly suppressed. In a typical experiment, solvent mixing and return of the sample to the spectrometer required about 3 min.

The B → Z kinetics were measured by the change in absorbance at 295 nm after a jump in NaCl concentration from 0.1 to 4.5 M NaCl or 3.25 M $NaClO_4$ (1). The initial relaxation

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*Present address: AT&T Bell Laboratories, 600 Mountain Avenue, Murray Hill, NJ 07974.
†To whom reprint requests should be addressed.

rate was obtained from a semilogarithmic plot of the change in absorbance vs. time.

RESULTS

Real-Time Solvent Exchange. Proton exchange in Z-DNA is sufficiently slow that it may be followed by real-time solvent exchange NMR. Fig. 1*A* shows an example of such an experiment on a 60-base-pair fragment of poly[d(G-C)] in 4.5 M NaCl at 4.7°C. After transfer to ^2H_2O, two slowly exchanging protons are detected at 13.1 ppm and at 8.5 ppm. The resonance at 13.1 ppm is due to the guanine imino protons (G_{im}) (21), while the spectral region between 6.5 and 9 ppm contains resonances from the nonexchangeable base protons (GH8 and CH6) and the cytosine amino protons (C_{am}). The intrinsic chemical shift of C_{am} is 1 ppm lower field than G_{am} and, due to the slow rotation about the C—N bond in cytosine, the C_{am} protons are usually resolved from each other (22, 23). On the basis of these chemical shift arguments, we assign the slowly exchanging proton at 8.5 ppm to the C_{am} that hydrogen bonds to the guanine carbonyl.

Due to the solvent suppression pulse sequence used, the intensities of the peaks depend not only on the number of protons but also on their frequency separation from the solvent peak. However, peaks close to each other in frequency will have similar intensities. Therefore, we have used the intensity of the nonexchangeable GH8 proton resonance at 7.8 ppm as an internal standard to estimate the intensity of the exchangeable proton resonance at 8.5 ppm. At the first time point in Fig. 1*A*, the intensity of C_{am} at 8.5 ppm is 85% of the intensity of GH8, but when extrapolated back to zero time, it has ≈95% of the intensity of GH8. Therefore, the C_{am} resonance at 8.5 ppm arises from one proton. This result indicates that there is little loss of intensity at 8.5 ppm during the time required to mix the sample with ^2H_2O and begin collection of spectra. In the experiment shown in Fig. 1*A*, the centerband frequency was located equidistant between the G_{im} and C_{am} resonances and, therefore, the two resonances are detected with comparable sensitivities. Comparison of these two resonances shows they have nearly equal intensities. The other three exchangeable protons (G_{am} and the other C_{am}) exchange too rapidly to be observed at this temperature and, because they resonate at higher field, they are strongly suppressed by the observation pulse.

Fig. 1*B* shows difference spectra in which the final (completely exchanged) spectrum was subtracted from the spectrum obtained at the time listed to the left. These difference spectra show that the protons with resonances at 13.1 and 8.5 ppm exchange at the *same* slow rate. This implies that the same transient fluctuation is responsible for the exchange of both slowly exchanging protons. The possibility that the slow exchange rates that we observe by NMR are artifacts due to association of the DNA in the relatively concentrated solutions (15 mg/ml) is unlikely in view of the following observations: (*i*) the rates of exchange observed from *solid* films of B- and Z-DNA are only a factor of 3 slower than those observed from dilute solutions (11); (*ii*) the slightly (2.4-fold) faster rates we observe for both the B → Z conversion and exchange, relative to values reported for dilute solutions (10), are in accord with the previously reported length dependence of the B →Z rate; (*iii*) the dipolar contribution to the spin-lattice relaxation rate (R_1) that we have measured for Z-DNA (13) (32 s^{-1} at 21°C in 4.5 M NaCl) is that expected for a 60-base-pair DNA in a solution with a viscosity of 2 centipoise instead of 1 centipoise (1 poise = 0.1 Pa·s) (much larger relaxation rates would have been observed if the DNA were highly aggregated); and (*iv*) R_1 is relatively insensitive to temperature up to 85°C.

Temperature Effects on Exchange. Fig. 2 is a semilogarithmic plot of the exchange of G_{im} protons as a function of time

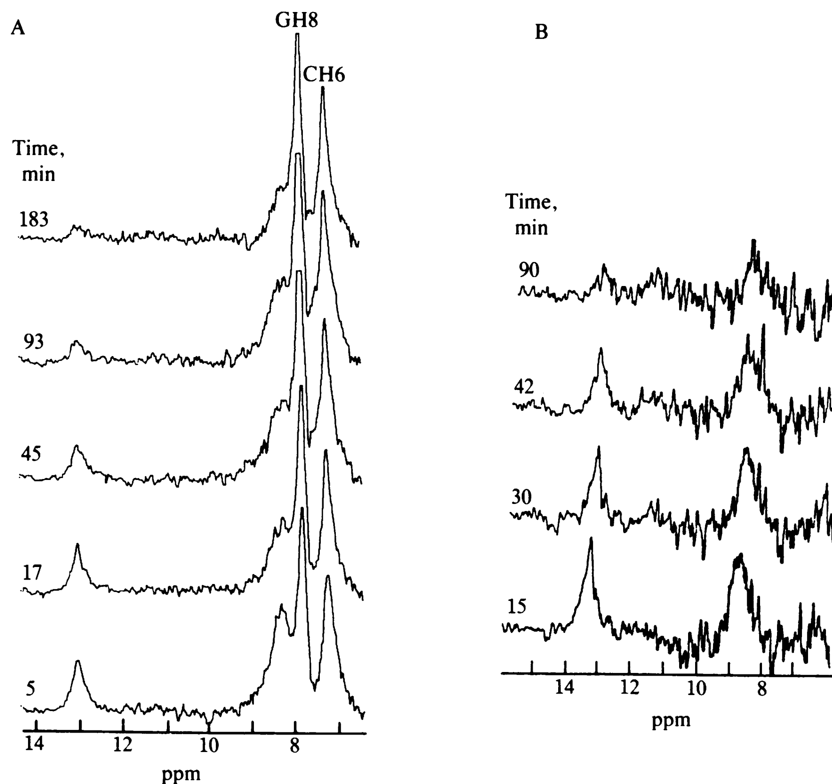


FIG. 1. 360-MHz ^1H NMR spectra showing the real-time solvent exchange of protons for deuterons in Z-form poly[d(G-C)] in 4.5 M NaCl at 4.7°C. The ppm are measured relative to 2,2-dimethyl-2-silapentane-5-sulfonate. (*A*) The dried DNA/salt mixture was dissolved in ^2H_2O at 0°C and placed in the NMR spectrometer, and the spectrum was measured as a function of time at 4.7°C. (*B*) Real-time solvent-exchange difference spectra in which the final (completely exchanged) spectrum was subtracted from the spectrum obtained at the time shown to the left of the spectra.

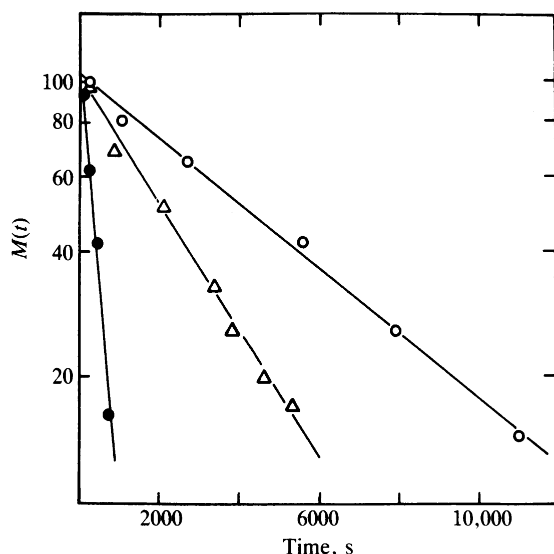


FIG. 2. Effect of temperature on the exchange of G_{im} protons measured by solvent exchange at 4.7°C (○), 11°C (△), and 25°C (●). $M(t)$ is the normalized G_{im} intensity at time t.

at several temperatures. Within experimental error, the exchange follows single-exponential kinetics. An Arrhenius plot of the exchange data (Fig. 3) yields an activation energy, E_a, for exchange of 21.6 ± 0.4 kcal/mol. Since the activation energy for the B → Z transition of poly[d(G-C)] in 4.5 M NaCl is also 22 kcal/mol (1), this suggested a correlation between the two phenomena.

Effect of $NaClO_4$ on Exchange. To determine whether the correlation between the B → Z kinetics and exchange from the Z form was simply coincidental, we examined the effect of 3.25 M $NaClO_4$ on G_{im} exchange. Conversion to the Z form occurs at lower concentrations of $NaClO_4$ vs. NaCl [1.6 vs. 2.5 M (1)] but, more importantly, the B → Z kinetics are strongly dependent on ionic strength in $NaClO_4$ but not in NaCl. We find that G_{im} exchange is accelerated by a factor of 5 relative to NaCl (see Fig. 3) although the E_a is apparently unchanged (23.0 ± 1.0 kcal/mol).

B → Z Kinetics. Previous studies have demonstrated that the B → Z kinetics of poly[d(G-C)] are sensitive to the type and concentration of ions present in solution, the temperature, and the helix length, but the E_a (and $\Delta H°$) for the B → Z conversion are not affected by changes in the salt concentration (1). Because the published values for the rate and E_a for the B → Z transition in high molecular weight samples were similar to those observed in our exchange experiments, we investigated the B → Z kinetics of the sonicated 60-base-pair poly[d(G-C)] fragments used in the NMR experiments. The rate of the B → Z transition is conveniently monitored by the change in absorbance at 295 nm after a jump in salt concentration from 0.1 M NaCl (B form) to 4.5 M NaCl (Z form) (1) or to 3.25 M $NaClO_4$. The result of this experiment are plotted in Fig. 3 along with the exchange results. In the 60-base-pair sonicated fragment, both the absolute rate and E_a for exchange of the G_{im} and for the B → Z transition are exactly the same, in two different solvent systems. In particular, the B → Z rate and the exchange rate are 5-fold faster in $NaClO_4$ than in NaCl. Despite the obvious differences in the two processes, one that is initiated from the B conformation and the other from the Z conformation, these results suggest there are common underlying factors controlling the rates for the two processes. The similarities between the B → Z transition and the differences between G_{im} exchange in B- and Z-poly-[d(G-C)] are summarized in Table 1. Note that the B → Z rates reported for high molecular weight samples (1) are about 1/2.5 times those we observed with shorter (≈60-base-pair) DNA.

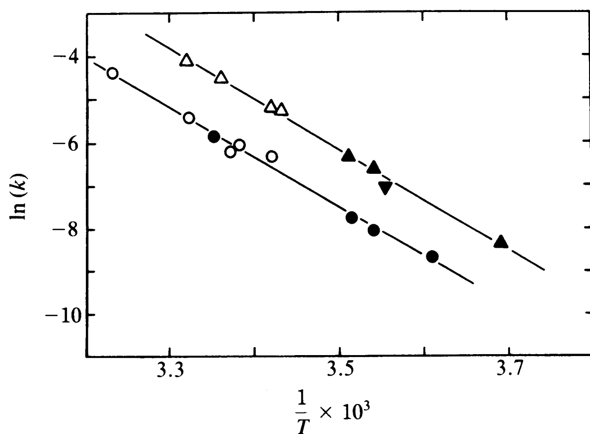


FIG. 3. Arrhenius plots of the solvent exchange and B → Z kinetics of sonicated poly[d(G-C)] in 4.5 M NaCl and 3.25 M $NaClO_4$. The units for k are s^{-1}. The data are for G_{im} solvent exchange (●) and the B → Z kinetics (○) in 4.5 M NaCl and the solvent exchange (▲) and B → Z kinetics (△) in 3.25 M $NaClO_4$. Note that in one experiment at 8.5°C ($1/T = 3.55 \times 10^{-3}$) in 3.25 M $NaClO_4$ the solvent also contained 0.05 M sodium phosphate (▼). This demonstrates that the G_{im} exchange is not catalyzed by buffer.

DISCUSSION

The fluctuations that result in exchange of the hydrogen-bonded imino and amino protons in Z-DNA are so slow that they can be followed by real-time solvent-exchange NMR. A key advantage of NMR over other exchange techniques [tritium exchange chromatography (23) and $^1H_2O/^2H_2O$ exchange monitored by UV (16, 17) and IR (11, 12) absorbance changes] is that the exchange rates of individual protons can be measured. The first unexpected result from our NMR study is that the two most slowly exchanging protons are the G_{im} and the hydrogen-bonded C_{am} protons. This assignment differs from that originally suggested (10) on the basis of tritium exchange and IR data and carries with it important implications with regards to the mechanism of exchange in Z-DNA and differences between the dynamics of B- and Z-DNA. Our revised assignment of the exchanging protons indicates that conformational fluctuations leading to the opening of base pairs are much less frequent than previously believed. For example, the time constant for exchange at 25°C from B-form poly[d(G-C)] (14) is 4.3 s, whereas in Z-form (4.5 M NaCl) it is 384 s. Thus, conversion from B- to Z-DNA reduces the exchange rate by a factor of 90 at room temperature, despite the fact that the activation energies of exchange for the two processes are nearly identical (21.6 ± 0.4 vs. 20.1 ± 0.4 kcal/mol).

Chemistry of Proton Exchange. To put the exchange properties of Z-DNA in perspective we briefly comment on pre-

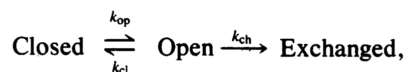
Table 1. Comparison of the rates of imino proton exchange and the B → Z transition in poly[d(G-C)]

Measurement*	Buffer†	E_a, kcal/mol	k (25°C), s^{-1}
Imino exchange (Z-DNA)	4.5 M NaCl	21.6 ± 0.4	0.0026
B → Z kinetics	4.5 M NaCl	23.0 ± 1.0	0.0028
Imino exchange (Z-DNA)	3.25 M $NaClO_4$	21.4 ± 2.1	0.011
B → Z kinetics	3.25 M $NaClO_4$	20.8 ± 0.4	0.016
Imino exchange (B-DNA)	0.1 M NaCl	20.1 ± 0.4	0.45

*The imino exchange in B-DNA was measured by the exchange contribution to G_{im} spin-lattice relaxation (14).

†The buffer also contained 0.01 M sodium cacodylate at pH 7.

vious analysis of exchange in RNA and DNA. Exchange of G and C protons is usually analyzed by the following scheme (17, 23, 24)



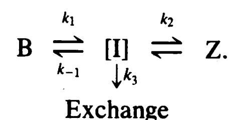
$$\text{Closed} \underset{k_{cl}}{\overset{k_{op}}{\rightleftharpoons}} \text{Open} \xrightarrow{k_{ch}} \text{Exchanged},$$

in which "closed" represents the exchange-inaccessible state, "open" represents the transiently formed solvent-accessible state, k_{op} and k_{cl} are the helix opening and closing rates, and k_{ch} is the rate of exchange from some state in which the exchangeable base protons are exposed to solvent. Exchange of G_{im} can occur only from the open state, whereas exchange from G_{am} may occur from the closed base-paired state (24). The effect of added catalysts on exchange may be used to determine the relative magnitudes of k_{op}, k_{cl}, and k_{ch} (17). The intrinsic exchange rate of G_{im} in the free base at room temperature and neutral pH is 500 s^{-1} (16); this is much faster than k_{cl} measured in stable DNA and RNA (16, 23, 24), and the exchange is in the opening-limited regime. Exchange of the two G_{am} protons occurs without base pair opening, presumably because exchange proceeds after protonation at GN3, which is exposed to the solvent in the intact base pair (24). Exchange of both C_{am} protons in RNA and DNA systems occurs at the same rate (12 s^{-1}, pH 7, 25°C) via transient opening of the G·C base pair and protonation at CN3 (24). The C_{am} exchange may be catalyzed by buffers but is pH independent (i.e., simultaneously acid and base catalyzed) between pH 6 and 8.

Proton Exchange in Z-DNA. Real-time exchange NMR shows that the two slowly exchanging protons in Z-DNA are the G_{im} and one of the C_{am} protons. Exchange of the slow protons cannot be catalyzed (25), indicating that G_{im} and C_{am} exchange are limited by the rate at which the base pairs open; all other protons can exchange from the closed state. Z-DNA is, therefore, the first DNA in which the two C_{am} protons are observed to exchange at very different rates and in which the G-imino is one of the most slowly exchanging protons. This unusual result is obtained because opening of G·C base pairs in Z-DNA is so slow that direct base catalysis becomes the rate-limiting step in the exchange of the exposed, non-hydrogen-bonded C_{am} proton. In normal DNA, the opening of base pairs is sufficiently fast that a pH-independent mechanism dominates the exchange (17, 23, 24). The fact that the hydrogen-bonded C_{am} proton is one of the two slowly exchanging protons further requires that rotation of the amino group about the C—N bond is slow (less than 2.6×10^{-3} s^{-1} at 25°C) compared to the base pair opening rate. Conversely, rotation of the G_{am} group must be relatively rapid, even in the base-paired state, to account for the fact that *both* of the G_{am} protons exchange more rapidly than the G_{im} proton. Since this mechanism can operate in both B- and Z-DNA, it accounts for the fact that the G_{am} exchange rate is the same in B- and Z-DNA (25, 26). Our interpretation of the exchange data also explains the surprising observation that catalysts accelerated the exchange of the three fastest exchanging protons in Z-DNA but not the two slowly exchanging protons (25).

The equilibrium population of open base pairs is typically calculated from comparison of the amino proton exchange rates in the stable double helix and the free base under the assumption that the intrinsic exchange rate from the exposed state is the same as from the free base (17, 23, 24). In the case in which the intrinsic exchange rate, k_{ch}, is less than k_{cl} (the preequilibrium regime), the fractional population of open base pairs is the ratio of the free base exchange rate to that in the intact DNA. Such an approach is not valid for Z-DNA because we cannot distinguish between the two possible pathways that result in exchange of the C_{am} proton resonating at 8.5 ppm: exchange of C_{am} because the closing rate is slow compared to the intrinsic exchange rate vs. rotation about the C—N bond in the exposed state.

Proton Exchange and the B → Z Transition. The remarkable correlation that we observe in 4.5 M NaCl between the B → Z transition and G_{im} proton exchange from Z-DNA could be entirely coincidental or might indicate that the two processes proceed through a common intermediate (open) state. The pronounced effect of $NaClO_4$ on the two rates supports the notion that the rate-limiting steps are similar in the two processes. A simple kinetic scheme that could account for our observation that the B → Z rate ($k_{B\text{-}Z}$) is identical with the exchange rate, k_{ex}, is as follows:



$$B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [I] \overset{k_2}{\rightleftharpoons} Z.$$
$$\downarrow k_3$$
$$\text{Exchange}$$

The only values for the various rate constants we found consistent with $k_{B\text{-}Z} \approx k_{ex}$ and $K = (Z)/(B) > 1$ were $k_3 > k_2 > k_{-1}$, in which case $k_{B\text{-}Z} = k_1 = k_{ex} = k_{-2}$, and $K = k_2/k_{-1} > 1$. This result is difficult to accept because it requires that the common intermediate state is formed from either the B or the Z form at the same rate and that the Z/B equilibrium is simply determined by the partitioning of the intermediate state back to Z or B. Other mechanisms involving more than one intermediate state can be constructed, but these do not lead to a necessary coupling of the B → Z rate and the exchange rate. The fact that the activation energies for the B → Z transition and exchange from Z-DNA appear to be nearly identical both in 4.5 M NaCl and in 3.25 M $NaClO_4$ suggests that entropic factors arising from changes in the hydration of DNA are important factors in determining the rates $k_{B\rightarrow Z}$ and k_{ex}.

The observations presented here are as follows: (*i*) The rate of base pair opening in Z-DNA is about 1/90th as fast as in B-form poly(dG-dC) at 25°C. (*ii*) The reduced rate of exchange in Z-DNA is due to a more unfavorable entropy of activation, since there is little difference in the apparent activation energy (21–22 kcal/mol) for exchange from B- and Z-DNA. (*iii*) Both the G_{am} protons and the non-hydrogen-bonded C_{am} proton exchange rates are 20 times faster than the G_{im} exchange rate. (*iv*) There is a remarkable correlation between the B → Z transition rate and the G_{im} exchange rate from Z-DNA that includes E_a, absolute rate, and salt effects. (*v*) In contrast with B-DNA, the G_{im} proton exchange rate in Z-DNA is dependent upon the nature and concentration of added salts.

We thank Dr. Neville Kallenbach and Dr. Thomas Jovin for helpful discussions regarding the exchange properties of Z-DNA and acknowledge the support of the National Science Foundation (Grant PCM83-03374).

1. Pohl, F. M. & Jovin, T. M. (1972) *J. Mol. Biol.* **67**, 375–396.
2. Wang, A. H. J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., Van Boom, J. H., Van der Marel, G. & Rich, A. (1979) *Nature (London)* **282**, 680–686.
3. Drew, H., Takano, T., Tanaka, S., Itakura, K. & Dickerson, R. E. (1980) *Nature (London)* **286**, 567–573.
4. Klysik, J., Stirdivant, S. M., Larson, J. E., Hart, P. A. & Well, R. D. (1981) *Nature (London)* **290**, 672–677.
5. Peck, L. J., Nordheim, A., Rich, A. & Wang, J. C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4560–4564.
6. Lafer, E., Moller, A., Nordheim, A., Stollar, D. & Rich, A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3546–3550.
7. Nordheim, A., Pardue, M., Lafer, M., Moller, A., Stollar, D. & Rich, A. (1981) *Nature (London)* **294**, 417–422.
8. Robert-Nicoud, M., Arndt-Jovin, D. J., Zarling, D. A. & Jovin, T. M. (1984) *EMBO J.* **3**, 721–731.
9. Nordheim, A., Lafer, E. M., Peck, L. J., Wang, J. C., Stollar, D. & Rich, A. (1982) *Cell* **31**, 309–318.

10. Ramstein, J. & Leng, M. (1980) *Nature (London)* **288**, 413–414.
11. Pilet, J. & Leng, M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 26–30.
12. Hartman, B., Pilet, J., Ptak, M., Ramstein, J., Malfoy, B. & Leng, M. (1982) *Nucleic Acids Res.* **10**, 3261–3279.
13. Mirau, P. A. & Kearns, D. R. (1983) in *Structure and Dynamics: Nucleic Acids and Proteins*, eds. Clementi, E. & Sarma, R. H. (Adenine, New York), pp. 227–239.
14. Mirau, P. A. & Kearns, D. R. (1984) *J. Mol. Biol.* **177**, 207–227.
15. Assa-Munt, N., Granot, J., Behling, R. W. & Kearns, D. R. (1984) *Biochemistry* **23**, 944–955.
16. Nakanishi, M. & Tsuboi, M. (1978) *J. Mol. Biol.* **124**, 61–71.
17. Mandal, C., Kallenbach, N. R. & Englander, S. W. (1979) *J. Mol. Biol.* **135**, 391–411.
18. Glickson, J. D., Phillips, W. D. & Rupley, J. A. (1971) *J. Am. Chem. Soc.* **93**, 4031–4038.
19. Johnston, P., Figueroa, N. & Redfield, A. G. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3130–3134.
20. Moore, P. & Kim, P. (1983) *Biochemistry* **22**, 2615–2622.
21. Kearns, D. R. (1977) *Annu. Rev. Biophys. Bioeng.* **6**, 477–523.
22. Shoup, R. R., Miles, H. T. & Becker, E. D. (1966) *Biochem. Biophys. Res. Commun.* **23**, 194–201.
23. McConnell, B. (1984) *J. Biomol. Struct. Dyn.* **1**, 1407–1421.
24. Teitelbaum, H. & Englander, S. W. (1975) *J. Mol. Biol.* **92**, 55–78.
25. King, H. D., Kallenbach, N. R. & Englander, S. W. (1983) *Biophys. J.* **41**, 421a.
26. Teitelbaum, H. & Englander, S. W. (1975) *J. Mol. Biol.* **92**, 79–92.