On the Metastability of Left-Handed DNA Motifs†

Ziv Reich,‡ Peter Friedman,‡ Yosef Scolnik,§ Joel L. Sussman,∥ and Abraham Minsky*,‡

Departments of Organic Chemistry, Materials and Interfaces, and Structural Biology, The Weizmann Institute of Science, Rehovot 76100, Israel

Received August 19, 1992; Revised Manuscript Received December 16, 1992

ABSTRACT: Alternating purine-pyrimidine DNA sequences such as poly[d(C-G)] or poly[d(m^5C-G)] undergo a cooperative, salt-induced structural transition from a right-handed B conformation, which prevails at relatively low ionic strength, into a left-handed Z form, generally believed to be stabilized by high salt concentrations. We report here that upon a monotonous increase of the ionic strength, the well-established B to Z transition is followed by a second, hitherto unobserved conformational change leading from Z-DNA back into a right-handed B-like form. This observation indicates that, in contrast with the current convention, the Z motif represents an unstable configuration relative to the B form at both *low and high* salt concentrations and that the occurrence of a left-handed DNA structure, presently depicted as a step function of the ionic strength, should rather be treated in terms of a pulse. The reported transition underscores the inherent metastability of the Z configuration, and indicates, consequently, that this motif is ideally suited to act as a structural regulatory element, as such an element should be endowed with a large susceptibility to cellular parameters.

The right-handed B-DNA and the left-handed Z-DNA represent two substantially different structural motifs that are in equilibrium with each other. At low salt concentrations, the Z-DNA is less stable than the B form, mainly because of a larger electrostatic repulsion between the phosphate groups on opposite strands of the double helix which are closer together in the Z motif than in the B-DNA (Rich et al., 1984). Under high ionic strength conditions, the B–Z equilibrium is shifted toward the left-handed conformation (Pohl & Jovin, 1972), due to a reduction of the hydration shells surrounding the phosphate groups (Saenger et al., 1986), as well as to an enhanced clustering of cations around the phosphates which results, in turn, in attenuated phosphate–phosphate repulsive interactions (Rich et al., 1984). Indeed, the notion that for alternating pyrimidine-purine sequences which can assume the Z configuration the left-handed motif would be favored over other DNA structures at high salt concentrations and would be further stabilized as the ionic strength is further increased has become generally established (Saenger, 1984).

Notably, attenuation of DNA hydration and of inter-phosphate repulsive interactions can be achieved not only under high ionic strength conditions but also through the interaction of nucleic acids with DNA-binding proteins, including, in particular, the positively-charged histones and protamines. This observation, in conjunction with the relative frequent occurrence of alternating pyrimidine-purine sequences in biological systems, should conceivably render the Z conformation into quite a ubiquitous motif. Yet, extensive efforts notwithstanding, direct evidence for the in vivo existence of Z-DNA is limited to exceedingly few cases (Nordheim et al., 1981; Jaworski et al., 1987; Rahmouni & Wells, 1989). How can such an apparent scarcity be reconciled with the general convention according to which the left-handed DNA configuration represents the stable, predominating structural motif for a rather broad range of cellular parameters?

The results presented in this study indicate that upon a continuous increase of the ionic strength, the well-established B to Z conformational change is followed by a second transition, namely, a Z to a B-like structure. This new transition, detected by means of circular dichroism, ^{31}P-NMR, and UV techniques, considerably limits the range of environmental conditions under which the left-handed DNA configuration is stabilized and provides, as such, a plausible and rather unexpected interpretation for the elusiveness of the Z form. Moreover, the existence of two sequential transitions that are affected by a monotonous alteration of the ionic strength suggests that the Z-DNA should be regarded as a structural switch that can straightforwardly be turned on and off and may, consequently, act as a very efficient structural control element in the regulation of genetic processes.

MATERIALS AND METHODS

The oligonucleotides specified under Results and Discussion were synthesized on an automated DNA synthesizer (Applied Biosystems). Following deprotection, the oligomers were purified by gel filtration on a Sephadex G25 (Pharmacia LKB Biotechnology, Inc.) column equilibrated with 20 mM NH_4HCO_3 (pH 8.0). DNA concentrations were determined by measuring the absorbance at 260 nm and applying the general relationship 1.0 OD = 33 μg/mL oligonucleotide. Annealing of the oligomers into double-stranded species was affected by heating the samples to 90 °C followed by a slow cooling. The purity of both oligonucleotides used in the study has been probed by means of a high-resolution electrophoresis (15% acrylamide gel) of the ^{32}P-end-labeled species against commercial 10-, 16-, and 20-mer double-stranded oligonucleotides.

Circular dichroism (CD) spectra were recorded on a Jasco J-500 spectropolarimeter equipped with a DP-500N data processor. Spectra were taken at room temperature, in 1-cm light path cells. Oligonucleotide concentrations were 5×10^{-5} M in base pairs. ^{1}H-Decoupled ^{31}P-NMR spectra were recorded on a Bruker AMX 400 at 318 K, using a 90° pulse and 2.0-s recycle time. A total of 18 000 transients were

† This work was supported by the Philip M. Klutznick Fund for Research at the Weizmann Institute of Science and by a grant from the Kimmelman Center for Biomolecular Structure & Assembly.

* Author to whom correspondence should be addressed. Incumbent of the Victor L. Ehrlich Career Development Chair.

‡ Department of Organic Chemistry.

§ Department of Materials and Interfaces.

∥ Department of Structural Biology.

0006-2960/93/0432-2116$04.00/0

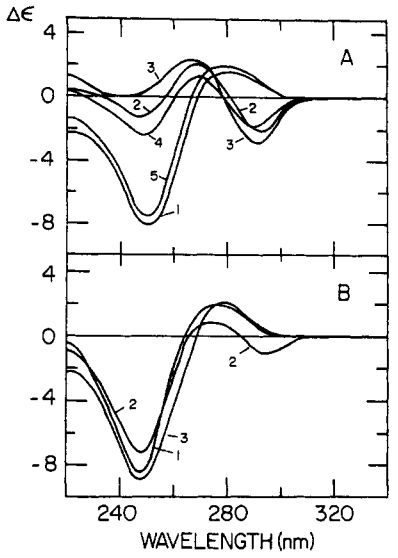

FIGURE 1: Circular dichroism spectra of the annealed forms of (A) d(C-G)$_8$ and (B) d(ATATATCGCGCGCGCG) as a function of $MgCl_2$ concentration. $MgCl_2$ concentrations were (panel A) (1) 0.1, (2) 1.0, (3) 2.0, (4) 2.8, and (5) 3.6 M and (panel B) (1) 0.8, (2) 1.8, and (3) 3.6 M. CD studies were executed on independently prepared samples as well as on a DNA sample in which the salt concentration has been gradually increased or decreased. For a given $MgCl_2$ concentration, identical spectra were obtained for the two sets of experiments.

accumulated, and an exponential line broadening of 10 Hz was applied. Chemical shifts were referenced to 85% phosphoric acid. UV spectra were recorded on a Hewlett-Packard 8400A diode array spectrophotometer at room temperature, using 1.0-cm light path cuvettes.

Sedimentation assays were conducted on 1-mL aliquots of the DNA segments at the same DNA concentration used for the CD experiments. $MgCl_2$ concentrations were 1.0, 1.5, 1.8, 2.0, 2.8, and 3.6 M. The samples were centrifuged in an Eppendorf microcentrifuge at 15 000 rpm (12000g) and their UV and CD parameters monitored before and after the centrifugation. Such conditions were shown to affect the sedimentation of Z*-DNA (Chaires & Norcum, 1988).

RESULTS AND DISCUSSION

The conformational changes sustained by the hexadecamer d(C-G)$_8$ upon exposure of the duplex to a progressive increase of ionic strength were monitored by means of circular dichroism, ^{31}P-NMR, and UV absorption techniques. The expected B to Z transition, completed at 2.0 M $MgCl_2$, is clearly indicated by an inversion of the optical properties (Pohl & Jovin, 1972) (Figure 1, panel A). As the salt concentration is raised above 2.0 M, a second structural transition is observed, leading to a DNA configuration whose optical parameters are virtually identical to those exhibited at low (0.1 M) $MgCl_2$ concentration (Figure 1A, spectra 3, 4, and 5). The notion that the duplex d(C-G)$_8$ undergoes two distinct salt-dependent conformational changes rather than one is strongly supported by ^{31}P-NMR. As the $MgCl_2$ concentration is increased, the single phosphate resonance which characterizes the B-DNA is split into two signals separated by 1.5 ppm (Figure 2, panel A). Such a splitting, presently accepted as a universal marker of the left-handed Z polymorph (Patel et al., 1979; Jovin et al., 1987), results from the alternating nature of the Z-DNA backbone. Upon a further increase of the ionic strength, the two ^{31}P peaks coalesce into a single resonance, indicating that in the high-salt DNA form, the backbone adopts a smooth, nonalternating configuration. The occurrence of two sequential transitions is further buttressed by the salient modifications of the A_{260}/A_{295} absorbance ratio that are observed as the $MgCl_2$ concentration is progressively increased. This ratio provides a sensitive measure of stacking interactions between base pairs and, since such interactions are significantly different in the B- and Z-DNA, of the B–Z equilibrium (Ramstein & Leng, 1980; Jovin et al., 1987). The bell-shape curve displayed by the A_{260}/A_{295} values as function of ionic strength indicates that the Z conformation predominates at 2.0 M $MgCl_2$ but is destabilized and totally negated as the salt concentration is further increased (Figure 3, curve 1).

A conspicuous similarity between the structural parameters of the conformation obtained from Z-DNA at high $MgCl_2$

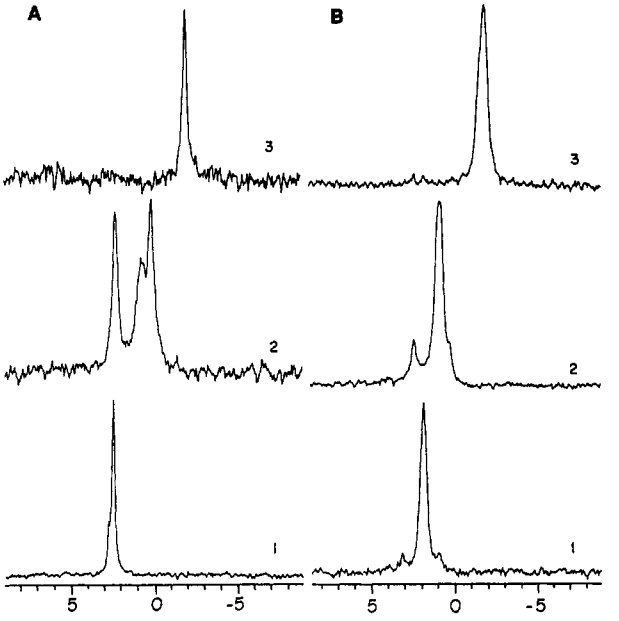

FIGURE 2: ^{31}P-NMR spectra of the annealed oligomers A and B (specified in Figure 1), recorded as a function of $MgCl_2$ concentration. Samples contained 0.5 mg/mL oligomer, 10% D_2O, and the following $MgCl_2$ concentrations: (panel A) (1) 0.1, (2) 2.0, and (3) 3.6 M; (panel B) (1) 0.8, (2) 1.8, and (3) 3.6 M. The samples were unbuffered, as the pH alterations affected by $MgCl_2$ at the concentrations used in the various experiments could not be effectively negated by buffers. The high-field chemical shifts observed upon the increase of the ionic strength result from a pH decrease from 6.8 (for 0.1 M $MgCl_2$ solution) to 6.1 (for 3.6 M solution). It should be emphasized that B–Z transitions were shown to be completely unaffected by a pH change in this range (Chen, 1984).

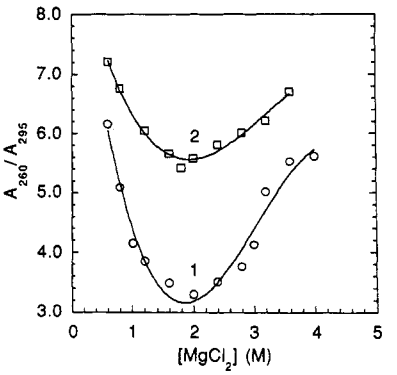

FIGURE 3: A_{260}/A_{295} absorbance ratios of the oligomers A and B (1 and 2, respectively) as a function of $MgCl_2$ concentration. As for the CD studies, each ratio was obtained from separately prepared samples, as well as from a given DNA sample in which the salt concentration has been gradually increased or decreased over the range of 0.8–4.0 M. Identical absorbance ratio curves were obtained by the two sets of experiments, indicating a complete reversibility.

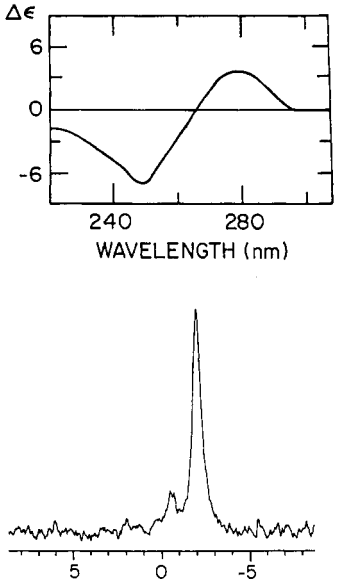

FIGURE 4: Circular dichroism and ^{31}P-NMR of the oligomer d(m^{5}C-G)$_8$ at 4.0 M $MgCl_2$. A right-handed, B-like configuration is indicated by both techniques.

concentrations and those characterizing the B form is pointed out. The circular dichroism spectra exhibited by the high-salt conformation and the B-DNA are nearly superimposable. Although the CD patterns in the 220–330-nm range do not allow an absolute assignment of the DNA handedness, they strongly suggest that the high-salt structure assumes a right-handed configuration reminiscent of the B polymorph rather than of the A or C motifs, which are characterized by CD spectra whose shapes are significantly different (Ivanov et al., 1973). The single phosphate NMR resonance displayed by the reported high-salt form is conceivably interpreted in terms of a nonalternating sugar–phosphate backbone which resembles that of the B configuration (Gorenstein et al., 1982). Notably, all known *left-handed* DNA conformations (i.e., Z, Z′, Z_{II}, or the aggregated Z* form) are characterized by an alternating backbone and are expected, consequently, to exhibit two distinct ^{31}P-NMR signals. The similar absorbance ratios exhibited by the low- and high-salt DNA conformations at the two extremes of the A_{260}/A_{295} bell-shaped curve indicate that similar, B-type stacking interactions between base pairs are operative within both forms. On the basis of these observations, it may be suggested that the conformation obtained from the Z motif at high ionic strength is a closely related structural variant of B-DNA and will, accordingly, be designated as B*.

DNA sequences that reveal a segmental B to Z transition and include, consequently, a junction such as the duplex d(ATATATCGCGCGCGCG) in which only the $(CG)_6$ segment can assume a left-handed configuration (Ellison et al., 1986; Dang et al., 1990) are found to undergo a segmental Z to B* transition as well (Figures 1B, 2B, and 3, curve 2). This observation indicates that the Z to B* conformational change is not affected by neighboring B-DNA segments or by the presence of a contiguous B–Z junction. The salt-induced transition from the Z polymorph to the B-like form is also detected in sequences in which the Z conformation is stabilized, i.e., the hexadecamer d(m^{5}C-G)$_8$ (Behe & Felsenfeld, 1981; Behe et al., 1981; Fujii et al., 1982; Zacharias et al., 1988); higher $MgCl_2$ concentrations are, however, required in order to allow the completion of the process in this case (Figure 4). The Z to B* transition is found to be induced also by $CaCl_2$ (at similar concentrations as $MgCl_2$) but not by the monovalent NaCl (data not shown).

A distinctive characteristic of Z-DNA is its propensity to aggregate into higher order structures, coined Z*-DNA, that can be sedimented at relatively low gravitational fields (Sande & Jovin, 1982; Sande et al., 1982; Castleman & Erlanger, 1982; Chaires & Norcum, 1988). Although the UV and CD parameters of the Z*-DNA are similar to those revealed by the Z motif (Sande & Jovin, 1982), the possibility that the spectral properties of the high-salt species reported in this study might originate from a distorted Z*-aggregated form had to be assessed. Toward this aim, the DNA segments at various $MgCl_2$ concentrations (specified under Materials and Methods) were subjected to a sedimentation assay conducted at those conditions shown to affect sedimentation of Z*-DNA forms (Chaires & Norcum, 1988). The CD and UV properties of the DNA segments before and after the centrifugation were found to be identical, strongly suggesting that no aggregated Z* species are formed. Moreover, it has been shown that Z*-DNA is induced and stabilized at elevated temperatures (Sande & Jovin, 1982). Thus, DNA samples at 2.0 M $MgCl_2$, characterized by Z-DNA parameters, were heated to 60 °C for 30 min, and the salt concentration was then adjusted to 3.6 M. The Z to B* transition, observed at room temperature, was detected under those conditions as well. Similarly, heating the 3.6 M $MgCl_2$–DNA solutions followed by dilution to 2 M resulted in a B* to Z conformational change. These observations indicate that the reported transitions are not temperature-dependent and that aggregated forms are not involved. Since the formation of left-handed aggregated forms was found to be crucially affected by the length of the DNA sequences (Chaires & Norcum 1988), the absence of such forms in the present study might be attributed to the short length of the studied segments.

Significantly, the sequential B–Z–B* conformational changes are found to be fully reversible: a backward process, namely, a B* to Z followed by a Z to B transition, is observed upon a gradual decrease of the $MgCl_2$ concentration. Such a complete reversibility and lack of hysteretic behavior, in conjunction with the observations that neither Tyndall scattering (derived from the A_{260}/A_{320} ratio) nor A_{260} hyperchromism is detected during the Z to B* transition, further indicate that the process is not associated with DNA aggregation or with strand separation but represents a real transition between two secondary structural motifs. The general consent according to which high ionic strength conditions merely induce and stabilize the Z-DNA is, consequently, challenged by our observations and should be revaluated; a more appropriate way to describe the salt-induced conformational changes exhibited by alternating pyrimidine-purine sequences is B $\rightleftharpoons$ Z $\rightleftharpoons$ B*.

Indeed, theoretical calculations based upon the potential-of-mean-force formalism have pointed toward a *right-handed* alternating B form as the most stable DNA motif at high salt (NaCl) concentrations (Soumpasis et al., 1987). The non-alternating conformation indicated by the single ^{31}P-NMR signal of the high-salt species described in this study might be related to the observation that a distinct backbone alternation within right-handed DNA motifs has been detected only for $d(AT)_n$ sequences (Viswamitra et al., 1982; Eckstein & Jovin, 1983); a different, but still a right-handed B-like, conformation might characterize the high-salt forms of the $d(CG)_n$ segments.

The equilibrium between right- and left-handed DNA polymorphs, as well as between different members of the Z

family, has been shown to be crucially affected by ion–DNA interactions (Saenger, 1984; Drew et al., 1980; Crawford et al., 1980; Wang et al., 1981; Gessner et al., 1985, 1989). Site-specific binding of ions has been invoked to explain transitions between members of a given DNA family, such as A to A′ (Arnott et al., 1968), B to C (Arnott & Selsing, 1975), or Z to Z′ (Drew et al., 1980). The high-salt Z to B* conformational change reported here is proposed to represent an *interfamily* transition promoted by specific ion–DNA interactions that are induced and enhanced at high ionic strength conditions (Skerjanc & Strauss, 1968). The findings that the transition is also affected by $CaCl_2$ but not by NaCl—for which only delocalized ion–DNA interactions are observed (Manning, 1978)—further support the notion that this conformational process is associated with specific binding effects and, conceivably, concomitant site-specific dehydration.

The functional significance of the Z-DNA remains a provocative and controversial issue. Several observations raise, however, the possibility that left-handed motifs might be involved in the regulation of biological processes either directly by acting as potential templates for RNA polymerase (Sande & Jovin, 1982; Hipskind & Clakson, 1983; Nordheim & Rich, 1983) and by modulating strand exchange (Fishel et al., 1988), thus affecting transcription and recombination processes, or indirectly by altering the extent of DNA packaging (Castleman et al., 1984; Thomas & Bloomfield, 1985; Reich et al., 1991) and supercoiling (Peck at al., 1982; Rich et al., 1984). Evidently, if the Z-DNA motif is indeed displaying regulatory effects, it should be characterized by a large sensitivity to cellular parameters. Such sensitivity has been indicated by the Z to B transition affected by various DNA-binding drugs (Chen et al., 1983; Walker et al., 1985), as well as by hydrostatic pressure (MacGregor, 1988), and is further underlined by our results which indicate that the Z conformation is a structural transient whose formation and undoing represent two events along a continuous alteration of the ionic strength. The elusiveness of Z-DNA, for which the reported results provide a hitherto unconsidered interpretation and point toward a rather unexpected source, seems to render this conformation into an ideal candidate to serve as a structural control element.

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