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Biochemistry

B- to Z-DNA transition probed by oligonucleotides containing methylphosphonates

(nucleic acid analogs)

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ABSTRACT **The simulation of the B–Z-DNA transition by using space-filling models of the dimer d(C-G) shows the possibility of hydrogen-bond formation between the N-2 amino group of the partially rotated guanine and one of the 5′-phosphate oxygens of deoxyguanylic acid. To probe the importance of this postulated interaction, analogs of the hexamer $d(C-G)_3$ were synthesized. These analogs contained a methylphosphonate linkage, of distinct stereochemistry, which replaced the first 5′-phosphate linkage of deoxyguanosine. The CD spectra in high salt concentration showed that the hexamer containing a methylphosphonate linkage with the R_P stereochemistry formed Z-DNA to the same extent as $d(C-G)_3$, whereas the hexamer containing a methylphosphonate linkage with the S_P stereochemistry did not form Z-DNA. These results are consistent with a mechanism in which an interaction between the N-2 amino group of guanine and the prochiral S_P oxygen of deoxyguanosine 5′-phosphate kinetically controls the formation of Z-DNA. A water bridge between the N-2 amino group of guanine and the 3′-phosphate oxygen of deoxyguanylic acid has been implicated in the stabilization of Z-DNA. To probe the importance of this water bridge, two additional analogs of the hexamer $d(C-G)_3$ were synthesized. These analogs contained a methylphosphonate linkage, of distinct stereochemistry, that replaced the first deoxyguanosine 3′-phosphate. The CD spectra showed that the hexamer containing a methylphosphonate linkage of the R_P stereochemistry underwent the transition to Z-DNA to the same extent as $d(C-G)_3$, whereas the hexamer containing a methylphosphonate linkage of the S_P stereochemistry underwent the transition to Z-DNA to a 35% lesser extent. Thus the water bridge involving the prochiral S_P oxygen provides modest stabilization energy for Z-DNA. These studies, therefore, suggest that the B–Z-DNA transition is regulated both thermodynamically and kinetically through hydrogen-bond interactions involving phosphate oxygens and the N-2 amino group of guanine.**

Pohl and Jovin (1) showed that the alternating copolymer poly[d(C-G)] underwent a salt-induced conformational change. The new species thus formed was left-handed Z-DNA, as subsequently shown by Wang *et al.* (2) from the crystal structure of $d(C-G)_3$. Further studies with alternating copolymers showed that poly[d(I-C)], poly[d(A-T)], and poly(dG)·poly(dC) do not form Z-DNA (1), while poly[d(G-T)]·poly[d(A-C)] appears to undergo the B–Z-DNA transition (3). Therefore, Z-DNA formation should be favored by an alternating purine-pyrimidine backbone, in which the 2-amino group of a purine is present on at least one of the strands of the duplex DNA. Further evidence that the 2-amino group of a purine is necessary for B–Z-DNA transition has been provided by Gaffney *et al.* (4), who showed that the hexamer $d(T\text{-}2NH_2A)_3$ displays a B–Z transition in high-salt solutions.

Although crystal structures of the B (5) and Z (2) conformations have revealed many features of the two structures, they have not defined the nature of the interactions that bring about Z-DNA formation in high-salt or organic-solvent solutions. Several theories have been put forth to explain the B–Z-DNA transition on the basis of particular interactions that are present in B-DNA or Z-DNA. All of these theories postulate either thermodynamic destabilization of B-DNA or stabilization of Z-DNA. Wang *et al.* (2) postulated, from the crystal structure of an oligonucleotide having a Z-DNA-like conformation, that a *syn*-anchoring water bridge involving the 2-amino group of guanine and one of the deoxyguanosine 3′-phosphate oxygens may be an important stabilizing interaction that allows Z-DNA to exist. Dickerson *et al.* (6) have postulated, from the crystal structure of an oligonucleotide having a B-DNA-like conformation, that the disruption of a spine of hydration in the minor groove of B-DNA by the 2-amino group of guanine results in the destabilization of B-DNA and hence the transition to Z-DNA. Other theories stress the importance of ionic shielding in Z-DNA because of the reduced distance between the deoxyguanosine 5′-phosphate groups in Z-DNA compared to the phosphate groups in B-DNA (1, 2).

The transition entails the rotation of the deoxycytidine base and sugar with respect to the phosphate backbone, accompanied by the rotation of the deoxyguanosine base about its glycosidic linkage (2). Space-filling models of the dinucleotide d(C-G) (Fig. 1) showed that during the B–Z transition, the partially rotated deoxyguanosine residue readily forms a direct hydrogen bond between the N-2 amino group of guanine and one of the deoxyguanosine 5′-phosphate oxygens. These hydrogen-bond interactions may substantially lower the potential energy barrier of the B–Z transition. To examine the role of interactions between the N-2 amino group and one of the deoxyguanosine 5′-phosphate oxygens in the formation of Z-DNA, we have employed oligonucleotides containing a methylphosphonate linkage in place of the phosphate moiety. Methylphosphonate linkages in oligonucleotides are neutral and are therefore suitable for probing charge–charge interactions involving DNA. Furthermore, oligonucleotides containing a methylphosphonate linkage of distinct stereochemistry allow the study of specific interactions involving phosphate oxygens. For these reasons, two diastereomeric hexanucleotide analogs of the hexamer $d(C-G)_3$ were synthesized. These analogs contained a methylphosphonate linkage of distinct stereochemistry,

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Abbreviations: C′, *N*-benzyl-2′-deoxycytidine; G′, *N*-isobutyl-2′-deoxyguanosine; Bzl, benzyl; Dmt, dimethoxytrityl; $tBuPh_2Si$, *t*-butyldiphenylsilyl; p, *o*-chlorophenyl phosphotriester.

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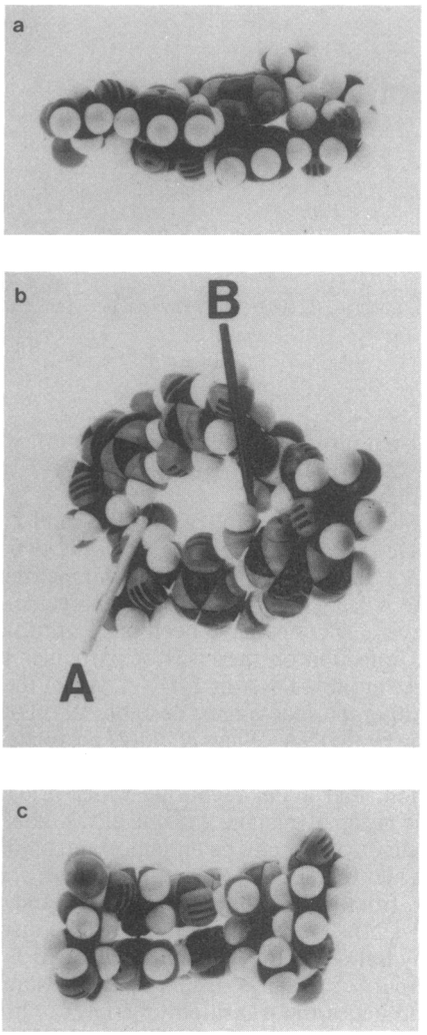

FIG. 1. Standard space-filling models of the B and Z forms of d[Cp(CH_3)G]-S_P-d(C-G) [p(CH_3) indicates a methylphosphonate linkage]. (*a*) View of the major groove of B-DNA in the plane of the bases. (*b*) An intermediate form between B- and Z-DNA. Arrow A points to a contact between the prochiral S_P oxygen and the N-2 amino group of deoxyguanosine; arrow B points to a contact between the methyl group of the (*S*)-methylphosphonate linkage and the N-2 amino group of deoxyguanosine. (*c*) View of minor groove of Z-DNA in the plane of the bases.

which replaced the first deoxyguanosine 5′-phosphate. Two additional diastereomeric hexanucleotide analogs of d(C-G)$_3$ were synthesized. These contained a methylphosphonate linkage in place of the first deoxyguanosine 3′-phosphate. Results of the studies with these four hexanucleotides are presented in this paper. On the basis of these results and previously published data, a mechanistic model is proposed to account for the salient features of the B–Z-DNA transition.

MATERIALS AND METHODS

Chemicals. Amino-protected nucleosides were purchased from Cruachem (Bend, OR). *N*-Methylimidazole, mesitylene sulfonyl chloride, and dimethoxytrityl (Dmt) chloride were purchased from Aldrich. Methylphosphonate dichloride was synthesized according to a published procedure (7).

Synthesis, Deprotection, and Purification of Hexanucleotides. The hexanucleotides were synthesized by using the phosphotriester method. The coupling reagent used in most of these syntheses was mesitylene sulfonyl chloride in conjunction with *N*-methylimidazole (8). The dinucleotides C′(Dmt)p(CH_3)G′(tBuPh_2Si) and G′(Dmt)p(CH_3)C′(tBuPh_2Si) were synthesized by coupling the 5′-protected nucleoside phosphonate with the 3′-silyl-protected nucleoside (C′, *N*-benzyl-2′-deoxycytidine; G′, *N*-isobutyl-2′-deoxyguanosine). The resulting dinucleotide diastereomers were separated by silica gel chromatography using stepwise increments of methanol [0.5%, 1%, 1.5%, 2% (vol/vol)] in methylene chloride containing 1% (vol/vol) pyridine as the eluate. Dimers were then desilylated with tetrabutylammonium fluoride, phosphorylated, purified by silica gel chromatography, and coupled with appropriately protected C′ṗG′ṗC′ṗG′(Bzl) to give the hexanucleotide C′(Dmt)p(CH_3)-G′ṗC′ṗG′ṗC′ṗG′(Bzl) (ṗ, *o*-chlorophenyl phosphotriester). By following a similar strategy, each stereoisomeric hexanucleotide C′(Dmt)ṗG′p(CH_3)C′ṗG′ṗC′ṗG′(Bzl) was synthesized. Experimental details of the synthesis will be described elsewhere.

The phosphate-protecting groups were removed by treatment with 2-oxopropional-1-oxime (9). Amino-protecting groups were removed according to Miller *et al.* (10). The 5′-Dmt hexanucleotides were purified by HPLC on a Microsorb C_{18} column (0.46 × 10 cm; Rainin Instrument, Woburn, MA), using a 0–50% (vol/vol) acetonitrile gradient. The Dmt group was then removed by treatment with 80% (vol/vol) acetic acid, and the completely deprotected oligonucleotide was finally purified by HPLC.

Characterization and Physical Studies of Hexanucleotides. Characterization was done by digesting the hexanucleotides with snake venom phosphodiesterase followed by alkaline phosphatase (Worthington). Resulting ratios of nucleosides to dimer or trimer were determined from the elution profile of the digestion mixture, using a Microsorb C_{18} column (0.46 × 25 cm), with a 0–15% (vol/vol) acetonitrile gradient. CD spectra were recorded on a Cary 60 CD spectrophotometer.

Crystallization and Structure Determination of the d[Cp(CH_3)G] Dinucleotides. Each isomer of d[Cp(CH_3)G] was purified by HPLC, and crystals were grown from 0.2 M triethylammonium acetate. Crystals of d[Cp(CH_3)G] peak 1 were found to be suitable for crystal structure determination. The crystals are tetragonal, space group $P4_12_12$, having unit cell parameters a = 19.260 Å, and c = 30.355 Å, with two dinucleotides and some solvent in the asymmetric unit. X-ray diffraction data were collected on a Syntex $P\bar{1}$ diffractometer using Cu K_α radiation at low temperature. A trial solution was obtained by direct methods and has been partially refined (R = 0.15). The two symmetry-independent dinucleotides form a duplex with a B-DNA-like conformation. Both phosphonate methyl groups are clearly identifiable; the phosphonate has the S_P configuration. Refinement is incomplete, as is development of a model of the solvent structure, which appears to be partially disordered.

RESULTS AND DISCUSSION

The chromatographic separation of the fully deprotected dinucleoside methylphosphonates by HPLC resulted in two peaks, as shown in Fig. 2 for d[Cp(CH_3)G]. Peak 1 was designated d[Cp(CH_3)G]-1, and peak 2 as d[Cp(CH_3)G]-2. Similar elution profiles were obtained for d[GP(CH_3)C] (data not shown). The stereochemical configuration of these two isomers was deduced from the CD spectral studies and the three-dimensional crystal structure of one of the isomers, d[Cp(CH_3)G]-1. Comparison of the CD spectra of the methylphosphonate dimers with those of the corresponding dinucleotides, as shown in Fig. 3, reveals that methylphosphonate dimers have the same CD band shape as their parent dinucleotides but have altered molar ellipticities. This difference in molar ellipticity indicates that each dimer has a different degree of base stacking in solution. Comparison of our CD profiles with those of Miller *et al.* (11) shows that the dimers d[Ap(CH_3)A]-1, d[Ap(CH_3)A]-2, d[Ap(CH_3)T]-1, and

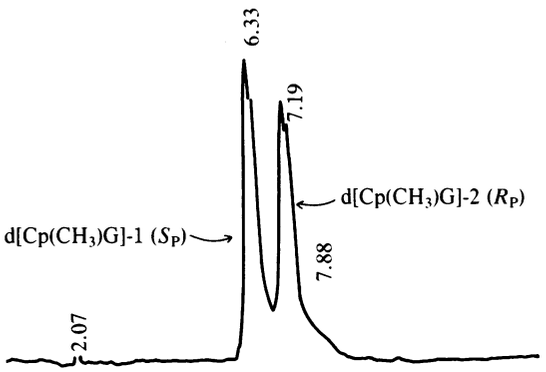

FIG. 2. HPLC elution profile of the dinucleotide d[Cp(CH_3)G] diastereomers. The isomers were separated by HPLC on a Microsorb C_{18} column (0.46 × 25 cm) and a 10-min gradient of 5–12.5% (vol/vol) acetonitrile in 0.1 M triethylammonium acetate (pH 7) at a flow rate of 1.5 ml/min.

d[Ap(CH_3)T]-2, which eluted from a silica gel column in the same numerical order as our d[Cp(CH_3)G] and d[Gp(CH_3)C] isomers, are qualitatively similar, namely in having more intense CD bands from isomer 1 in each case. The order of elution from a reverse-phase HPLC column is consistent with this: the isomer first eluted exhibits a more intense CD pattern than does the other isomer. Model-building studies (Fig. 1) and Miller *et al.* (11) have shown that when methylphosphonate dimers stack in a normal B-DNA-like configuration, the S_P isomer tends to be better stacked and therefore less hydrophobic than the R_P isomer. For these reasons, we tentatively assigned the S_P stereochemistry to the dimers d[Cp(CH_3)G]-1 and d[Gp(CH_3)C]-1. Correspondingly, R_P stereochemistry was assigned to the dimers d[Cp(CH_3)G]-2 and d[Gp(CH_3)C]-2. Conclusive evidence that the dimer d[Cp(CH_3)G]-1 has the S_P configuration has emerged from the three-dimensional crystal structure. These crystal-structure studies clearly show the methyl group in a pseudoaxial (S_P) position with bases being Watson–Crick

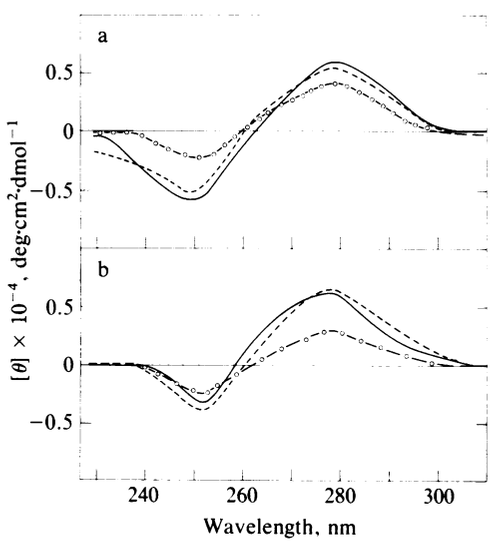

FIG. 3. CD spectra of methylphosphonate dimers and the corresponding dinucleotides. (*a*) —, d(C-G); ---, d[Cp(CH_3)G]-1; -○-, d[Cp(CH_3)G]-2; (*b*) —, d(G-C); ---, d[Gp(CH_3)C]-1; -○-, d[Gp(CH_3C]-2. Both d[Cp(CH_3)G]-1/d[Cp(CH_3)G]-2 and d[Gp(CH_3)C]-1/d[Gp(CH_3)C]-2 are diastereomeric pairs that differ in stereochemistry about the methylphosphonate linkage. All spectra were recorded in 0.1 M NaCl/0.01 M sodium cacodylate, pH 7, at an A_{260} of 1.30 at 25°C. The suffixes 1 and 2 refer to the order in which the fully protected dimers were eluted from the silica gel column.

base paired in a B-DNA-like conformation (details will be published elsewhere). The crystal structure of the other isomer, d[Cp(CH_3)G]-2, has not yet been determined. Although the stereochemical assignments for d[Gp(CH_3)C]-1 and d[Gp(CH_3)C]-2 are tentative, they are likely to be correct, on the basis of their characteristic CD pattern.

Two pairs of diastereomeric hexanucleotides were synthesized by using these methylphosphonate dimers of defined stereochemistry. All four hexanucleotides, d[Cp(CH_3)GpCpGpCpG] (CG-*S*), d[Cp(CH_3)GpCpGpCpG] (CG-*R*), d[CpGp(CH_3)CpGpCpG] (GC-*S*), and d[CpGp(CH_3)CpGpCpG] (GC-*R*), were judged to be nearly homogeneous by their HPLC elution profile (data not shown). Characterization of these hexanucleotides was by digestion with snake venom phosphodiesterase and alkaline phosphatase, followed by HPLC separation of the nucleoside and nucleotide products. Under our experimental conditions, snake venom phosphodiesterase (a 3′-to-5′ exonuclease) did not cleave through a methylphosphonate linkage. Digestion of CG-*S* and CG-*R* (Table 1) by snake venom phosphodiesterase and alkaline phosphatase should yield dC and dG, as well as the methylphosphonate dimer d[Cp(CH_3)G]. Similar digestion of GC-*R* and GC-*S* should yield dC and dG, as well as the trinucleotide d[CpGp(CH_3)C]. As shown in Table 1, the experimentally determined ratios of dC to dG to dimer or trimer for the four hexamers were in agreement with the expected ratios. In addition, the elution profile showed the presence of a single peak, corresponding to one of the diastereomeric dimers in the case of the hexanucleotides CG-*S* and CG-*R*, and one of the diastereomeric trimers in the case of the hexanucleotides GC-*S* and GC-*R*.

For monitoring B–Z transitions in DNA, CD spectroscopy has been a sensitive and reliable tool, especially for oligonucleotides containing alternating guanine and cytosine (1, 13, 14). We have therefore employed this technique to monitor the B–Z transition for each of the four hexanucleotides. The CD spectra of the hexanucleotides in high and low salt concentrations, determined under the experimental conditions employed by Uesugi *et al.* (13) for the hexamer d(C-G)$_3$, are shown in Fig. 4. In low salt, all four hexamers have a CD spectrum that is characteristic of B-DNA. This duplex structure shows exceptional stability for each of the four hexanucleotides, as indicated from the temperature dependence (data not shown). This indicates that a methylphosphonate linkage of either the R_p or S_p stereochemistry does not appreciably destabilize the B-DNA conformation of oligonucleotides containing C and G. The CD spectra of CG-*R* and GC-*R* in high salt (Fig. 4 *b* and *d*) show a negative band at 295 nm and a shift towards positive ellipticity in the band at 255 nm. This is characteristic of d(C-G)$_3$ shifting into the Z conformation, as shown by Uesugi *et al.* (13). The extent to which CG-*R* and GC-*R* undergo transition to Z-DNA is comparable to that of d(C-G)$_3$. On the other hand, GC-*S* (Fig. 4*c*) undergoes a salt-induced transition only to approximately 65% of the extent GC-*R* does (Fig. 4*d*). Finally, the CD spectrum of CG-*S* (Fig. 4*a*) does not show any indication of a salt-induced transition to Z-DNA. The slight change observed in the intensity of the bands is probably due to reduction of the helicity of B-DNA in high salt (13, 14).

A priori, a difference in the ability of two similar compounds to undergo a given transition can be due to either thermodynamic or kinetic factors. Thermodynamic factors can act by either stabilizing or destabilizing the B or Z conformations of DNA. Kinetic effects arise by either raising or lowering the potential-energy barrier for a given conformational change. In our experiments we did not attempt to measure the kinetics of the B–Z transition. Instead, we waited several hours between the addition of DNA to a high-salt solution and the measurement of the CD spectrum.

Table 1. Physical and structural properties of the hexanucleotides

Hexanucleotide (abbreviation)	Assigned stereochemistry at phosphonate	Equilibrium constant Z/B conformation*	Nucleoside to dimer or trimer ratio, as mol of nucleoside†		
			dC	dG	Dimer or trimer
d[Cp(CH_3)GpCpGpCpG] (CG-*S*)	S_P	—	2.0 ± 0.2	2.0 ± 0.2	1.9 ± 0.2
d[Cp(CH_3)GpCpGpCpG] (CG-*R*)	R_P	0.47	2.0 ± 0.2	2.2 ± 0.2	2.1 ± 0.2
d[CpGp(CH_3)CpGpCpG] (GC-*S*)	S_P	0.24	1.0 ± 0.2	2.1 ± 0.2	2.9 ± 0.2
d[CpGp(CH_3)CpGpCpG] (GC-*R*)	R_P	0.44	1.0 ± 0.2	2.0 ± 0.2	2.8 ± 0.2
$d(CpG)_3$	—	0.44‡	—	—	—

*Calculated by using $K = (\theta_{B_{295}} - \theta_{295})/(\theta_{295} - \theta_{Z_{295}})$. $\theta_{B_{295}}$ is the high-salt value of the ellipticity at 295 nm for CG-*S*. $\theta_{Z_{295}}$ is the expected high-salt value for the hexamer $d(CpG)_3$ when completely in the Z conformation. It was calculated by using the above equation and the equilibrium constant at room temperature given by Holak *et al.* (12) and the high oligonucleotide concentration value of θ_{295} in high salt given by Uesugi *et al.* (13). θ_{295} is the high-salt value of the ellipticity at 295 nm taken from Fig. 4.

†Elution of chromatograms was monitored at 254 nm. For deoxyguanosine, $\varepsilon_{254} = 13{,}600\ M^{-1}\cdot cm^{-1}$; for deoxycytidine, $\varepsilon_{254} = 6300\ M^{-1}\cdot cm^{-1}$. Additive absorbance values were used for the dimers and trimers. The given ratios are within 20% of the expected values.

‡Calculated from the low oligonucleotide concentration value of θ_{295} from Uesugi *et al.* (13).

Kinetic effects, however, are observed if the potential-energy barrier is raised to the point where the rate of B–Z transition is essentially zero at a given temperature. Thus, under our experimental conditions, thermodynamic factors are involved in changing the extent of Z-DNA formation, while kinetic effects can be invoked only to explain the complete lack of Z-DNA formation.

The ability of the hexanucleotides CG-*R* and GC-*R* to undergo B–Z transition in a high-salt solution, to approximately the same extent as $d(C\text{-}G)_3$, does show that charge–charge interactions involving the phosphate group, and interactions involving the R_P prochiral oxygen, are not essential for the B–Z transition to occur. Moreover, charge–charge interactions and other interactions involving the R_P prochiral oxygens do not appreciably alter the free energy difference between B- and Z-DNA at high ionic strength. These observations are in fact consistent with features of the crystal structures of B- and Z-DNA. The crystal structure of B-DNA (5) shows the prochiral R_P oxygen of the phosphate group to be in a relatively more hydrophilic portion of the helix than the prochiral S_P oxygen (Fig. 1*a*). The crystal structure of Z-DNA (2), on the other hand, shows the prochiral R_P oxygen of deoxyguanosine 5′-phosphate to be in a more hydrophilic environment than the prochiral S_P oxygen, while in the deoxyguanosine 3′-phosphate, the two nonesterified oxygens appear to be in regions of equal hydrophilicity within the Z-DNA helix. Thus destabilization, by forcing the methyl group of the (*R*)-methylphosphonate linkage into a hydrophilic environment, should not be an important factor in the transition to, or in the structure of, Z-DNA.

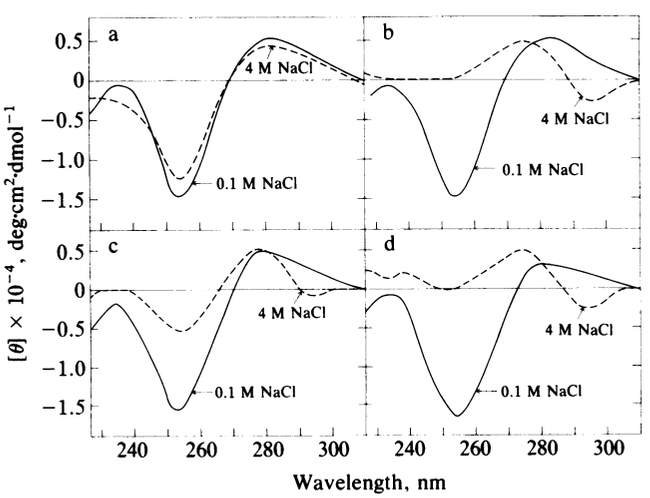

FIG. 4. CD spectra of diastereomeric pairs of hexadeoxynucleotides containing a methylphosphonate linkage. (*a*) CG-*S*; (*b*) CG-*R*; (*c*) GC-*S*; (*d*) GC-*R*. Spectra were recorded in 0.1 M NaCl (—) and 4 M NaCl (---), both containing 0.01 M sodium cacodylate, pH 7, at 25°C. Concentration of oligonucleotides was such that $A_{260} = 1$.

The two hexadeoxynucleotides with the S_P stereochemistry, CG-*S* and GC-*S*, differ in their ability to undergo the B–Z-DNA transition. Hexadeoxynucleotide GC-*S* underwent 65% of the transition to Z-DNA in high salt that GC-*R* did. This is indicative of an interaction involving the methyl group of the (*S*)-methylphosphonate linkage that either stabilizes B-DNA or destabilizes Z-DNA for the GC-*S* isomer. Indeed, this is consistent with the observation of Wang *et al.* (2) that a water bridge between the N-2 amino group of guanine and the prochiral *S* oxygen of the deoxyguanosine 3′-phosphate group provides a stabilizing interaction that helps to maintain the Z-DNA conformation. From the equilibrium constants given in Table 1 for GC-*R* and GC-*S*, the free energy of this water bridge can be estimated to be 0.2 kcal/mol of water bridge (1 kcal = 4.18 kJ).

Most interestingly, the hexamer CG-*S*, which contains a methylphosphonate linkage having the S_P stereochemistry at the 5′ position of the first deoxyguanosine, does not undergo any observable transition to Z-DNA. Thermodynamically, this could arise from extreme destabilization of Z-DNA, extreme stabilization of B-DNA, or a combination of the two. In fact, the crystal structures of B-DNA and Z-DNA show that the phosphate groups 5′ to the guanosine residues are only 12 Å apart in Z-DNA (2), compared to 17 Å in B-DNA (5). The lack of charge on a methylphosphonate linkage would tend to stabilize CG-*S* and CG-*R* in the Z-DNA conformation, by reducing the electrostatic repulsion between phosphate groups. These structures also show that the prochiral S_P oxygen of the 5′-phosphate group of guanosine is in a more hydrophobic environment than the prochiral R_P oxygens in both B-DNA and Z-DNA (Fig. 1 *a* and *c*, respectively). Hydrophobic interactions involving the phosphonate linkage should therefore have little effect on the B–Z equilibrium for the hexamer CG-*S*. Z-DNA is formed by dehydrating DNA, and a methylphosphonate linkage should dehydrate more readily than a phosphate linkage. For this reason, stabilization of B-DNA by prevention of dehydration seems unlikely. Thus, the structural and physical data on B-DNA and Z-DNA argue against a thermodynamic explanation for the absence of a B–Z transition in CG-*S*. The absence of the B–Z transition in CG-*S* should therefore be due to a kinetic effect.

In the B–Z transition, both the sugar and the base of deoxycytidine must rotate with respect to the phosphate backbone, while the base of deoxyguanosine rotates about the glycosidic linkage. The simulation of the B–Z transition using space-filling models of the dimer d(C-G)·d[Cp(CH_3)G]-S_P (Fig. 1*b*) indicates that, during rotation of the guanine residue about the glycosidic bond, the prochiral S_P oxygen of the 5′-phosphate group of deoxyguanylate should enter into a direct hydrogen-bond contact with the N-2 amino group of guanine. This phosphate oxygen–amino group contact should substantially lower the potential-energy barrier due to both hydrogen-bond factors and electrostatic factors. Since the methyl group in the S_P methylphosphonate linkage is unable to form a hydrogen bond, the potential-energy barrier of the B–Z transition is raised accordingly. This rise should be large enough, under our experimental conditions, to prevent Z-DNA formation for the hexamer CG-*S*.

On the basis of our results and the observations made by Dickerson and coworkers (5, 6) concerning specific hydration in B-DNA, which involves the N-2 amino group of guanine and unesterified phosphate oxygens, the following pathway for the B–Z transition can be put forth: These interactions involving water molecules are disrupted, allowing the formation of a kinetically important contact between the N-2 amino group and the prochiral S_P oxygen of the 5′-phosphate of deoxyguanylate. This hydrogen-bond interaction helps to compensate for the unstacking of the bases from the B-DNA conformation. The hydrogen-bond contact is then broken as the bases begin to stack in the Z-DNA conformation. A water bridge, which helps to stabilize Z-DNA, is ultimately formed between the N-2 amino group of guanine and the prochiral S_P oxygen of deoxyguanosine 3′-phosphate. The simulation of the B–Z transition with the trimer d(C-G-C)·d(G-C-G) (not shown) indicates that, when the base pair 3′ to the deoxyguanosine residue undergoing the B–Z transition is in the B-DNA conformation, substantial steric hindrance must be overcome to complete the rotation of the base pair into the Z conformation. Thus, when the base pair above or below the dimer undergoing the transition to Z-DNA is disrupted, or is already in the Z conformation, Z-DNA formation should be facilitated.

The above model contains several features previously postulated by Harvey (15), Sarma *et al.* (16), and Olson *et al.* (17)—namely, maintenance of Watson–Crick base pairing in the dimer undergoing transition (15–17), formation of a cavity to allow the transition to occur (15–17), and symmetrical flipping of base pairs through the high *anti* conformation of guanosine (15). Important new features in this model, however, help to explain the nature of the B–Z transition—namely, that the d(C-G) dimer undergoes the transition as a unit, that disruption of a base pair in the B conformation below the dimer undergoing the transition facilitates the B–Z transition, and, most importantly, that the direct hydrogen-bond contact between the N-2 amino group and the prochiral S_P oxygen of deoxyguanosine's 5′-phosphate linkage is an essential requirement for the transition to occur.

This pathway offers a facile explanation for the formation of Z-DNA under dehydrating conditions. Dickerson attributed the stability of B-DNA to a spine of hydration in the minor groove of DNA. Our model predicts that, to form Z-DNA, not only the "spine of hydration" but also specific hydration involving nonesterified phosphate oxygens must be disrupted. One is then tempted to speculate that the disruption of specific hydration involving nonesterified 5′-phosphate oxygens may well be the role played by the 5-methyl group in 5-methylcytosine, as well as polyvalent cations and the perchlorate anion, in enabling the B–Z transition to occur at reduced ionic strength (18). This pathway also suggests that poly[d(A-T)] and poly[d(I-C)] are kinetically prevented from forming Z-DNA. It implies that nucleation of the B–Z transition should occur at the ends in linear DNA. Intrachain nucleation would require the disruption of two base pairs, whereas nucleation at the ends of DNA requires the disruption of only one base pair. This is in agreement with the data of Pohl and Jovin (1), that the rate of the B–Z transition is proportional to the number of ends present and not to the number of base pairs in a strand. This pathway indicates, too, that disruption of base pairs adjacent to the dimer undergoing transition is less when the adjacent base pairs are in the Z conformation than when they are in the B conformation. This rationalizes the cooperativity of the B–Z transition (1) as well as the differences in the kinetics of the B–Z transition versus the Z–B transition (19).

The pathway also offers an explanation for the data of Jovin *et al.* (20), that Z-DNA formation was prevented by the chiral substitution of a sulfur for a nonesterified oxygen, as a sulfur atom forms a much weaker hydrogen bond than an oxygen. The recent observation of Kang *et al.* (21) that crosslinked Z-DNA cannot revert to B-DNA also supports the proposed pathway. Theoretical studies have begun to show that this direct hydrogen-bond contact between the N-2 amino group and the 5′-phosphate oxygen may well be involved in increasing the conformational flexibility of deoxyguanosine-containing sequences that seem unable to form Z-DNA.

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