Facile Interconversion of Duplex Structures Formed by Copolymers of d(CG)†

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ABSTRACT: Correlations between DNA sequence and reactivity have often been drawn with an implicit or explicit connection to duplex structure. An in vitro model using oligonucleotides of defined sequences has been developed to characterize a potential source of the hypersensitivity that naturally occurring regions of redundant sequence exhibit with many nucleases. S-1 nuclease was used here to diagnose the unusual hybridization of copolymeric DNA, $d(CG)_6$, and related oligomers, through product and kinetic analysis. Fully complementary but redundant sequences reacted with this enzyme almost an order of magnitude faster than did heterogeneous fragments of DNA. Hydrolysis products of the copolymers indicated that conformations with unpaired termini were the sole substrates under these studies, and only a facile equilibrium between aligned and extended structures was required to explain the heightened reactivity of this DNA. For example, $d(CG)_6$ was converted to $d(CG)_5$ and $d(CG)_4$ whereas $d(CG)_4C$ was initially processed to an octamer and then only later to a hexamer. Catalysis by S-1 exhibited no other substrate or product specificity; even the disordered bases in the loop region of a hairpin structure, $d(CG)_3T_4(CG)_3$, did not provide sites of enhanced enzyme action. The rate of DNA consumption under standard conditions was proportional to the expected concentration of overhanging sequences rather than the absolute amount of DNA present. All initial attempts to saturate enzyme activity failed, and therefore, the rate of substrate formation through strand slippage was always faster than the catalytic depletion of unpaired bases. Only a low-energy transition state(s) must then separate the various hybridized species since this structural equilibration proceeded readily under conditions of 10 mM potassium phosphate, pH 7, 100 mM NaCl, and 22 °C.

The conformational diversity of DNA is now the subject of numerous studies in order to gain a predictive understanding of polynucleotide structure and to define its role in gene expression, mutation, and repair. A number of very exotic complexes have recently been characterized (Wells, 1988; Barton, 1988), but even typical B-helix-forming regions of DNA have been shown to exhibit sequence-dependent deviations from an idealized or generalized form (Shakked & Rabinovich, 1986). Often, a variety of structures will coexist within a single polynucleotide duplex and consequently produce segments of DNA that are hypo- or hyperreactive to chemical and enzymatic modification. These unusual patterns of reactivity have now become diagnostic of and synonymous with the microheterogeneity of DNA structure. S-1 nuclease, only one of many structural probes, has been applied to the analysis of Z-helixes (Johnston & Rich, 1985), B/Z and Z/Z junctions (McLean & Wells, 1988), H-structures (Johnston, 1988), hairpin/cruciforms (Gough & Lilley, 1985), and cohering telomeres (Oka & Thomas, 1987). To elaborate the atomic details of each form of DNA, however, chemical modification studies of large polynucleotides must be replaced by crystallographic and magnetic resonance techniques directed at well-defined oligonucleotide models.

While studies on oligonucleotides have provided a wealth of structural information, complications can arise from such inherent characteristics as the unnaturally high ratio of duplex ends to internal base pairs (Luthman & Behe, 1988). Short sequences of alternating dC-dG are popularly used to delineate the chemical properties of B- vs Z-helixes, but such redundancies in sequence can also lead to a multiplicity of solution structures. Under ambient conditions, hybridization would ordinarily be expected to maximize hydrogen bonding between strands by forming a fully complemented duplex. Magnetic resonance studies have suggested that the core of duplex $d(CG)_6$ does form a B-like structure under low ionic strength yet the terminal residues remain distinctly disordered or highly flexible (Sheth et al., 1987). Other symmetric sequences have been shown to equilibrate between a duplex, a hairpin, and a variety of structural intermediates (Xodo et al., 1989). This interconversion is particularly common for redundant sequences based on $d(CG)_n$ (Xodo et al., 1988a) and $d(AT)_n$ (Scheffler et al., 1968).

The anomalous behavior of alternating copolymers also persisted when such sequences were inserted into plasmids (Suggs & Wagner, 1986; Evans & Efstratiadis, 1986). Redundant sequences in nature, often found near the beginning of certain genes, may similarly adopt an equilibrium of conformations (Hentschel, 1982; Mace et al., 1983; Weintraub, 1983; Fowler & Skinner, 1986). A pronounced feature of these regions is their unexpectedly high sensitivity to S-1 and other nucleases (Elgin, 1989). Depending on the exact sequence of each hyperreactivity tract of bases, alternative non-B, non-Z structures such as triple helical arrangements or slipped structures have been proposed to explain this behavior. In model studies described here, the unusually fast reaction between $d(CG)_n$ and S-1 nuclease can be explained by a facile equilibration between fully hybridized DNA and slipped structures that form the only target of enzyme action, dangling ends (eq 1).

Experimental Procedures

Materials

S-1 nuclease was purchased from Bethesda Research Laboratories, and the nucleotides dC, pdC, dG, pdG, and dCG were obtained from Sigma Chemical Co. Individual oligonucleotides were synthesized by standard solid-phase phos-

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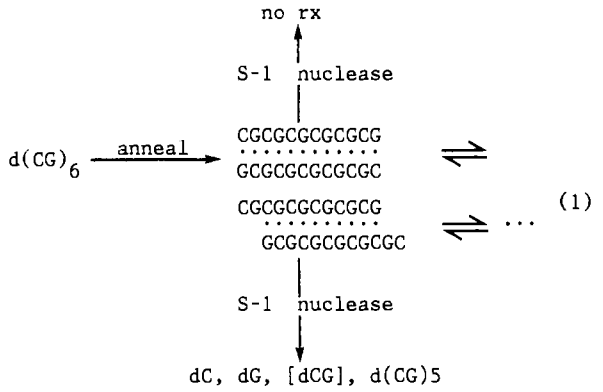


phoramidite chemistry and purified after complete deprotection by ion exchange chromatography under the strongly denaturing conditions of pH 12. Similar chromatographic procedures were also used to separate all products for identification and kinetic analysis (Figure 1A). All other reagents were of the highest commercial quality and used without further purification.

Methods

Sample Preparation and Nuclease Digestion. DNA hybridization was allowed to reach a thermodynamic equilibrium by placing solutions of 2–20 μM oligonucleotide, 10 mM potassium phosphate, pH 7, and 100 mM NaCl in a water bath at 90 °C. This bath was then immediately turned off and allowed to cool under ambient conditions. Once the bath (and samples) returned to room temperature (>3 h), enzymatic analysis was initiated by the addition of S-1 nuclease to a typical concentration of 5000 units/mL. These reaction conditions were designed more for physiological relevance than for maximizing enzyme turnover; the enzyme activity listed here was defined and measured under optimal conditions (pH 4.6, 37 °C, etc.) by the supplier and does not reflect the activity available in the illustrated studies at pH 7.

Rate and Product Analysis. The hydrolysis process catalyzed by S-1 nuclease was always maintained under ambient conditions and monitored by discontinuous analysis as represented in Figures 1–3. Aliquots of the reaction incubations were quenched into 1 mL of 11.5 mM NaOH, and each product and starting material was separated by anion exchange chromatography [Mono Q (Pharmacia)] using a gradient of NaCl (see Figure 1A). DNA was detected by its absorbance at 254 nm and quantified with an integrating recorder (Shimadzu C-R3A). Detector output was standardized against $d(CG)_6$ absorption under similarly denaturing conditions. Absorptivity values of all DNA sequences were estimated from the sum of individual nucleotides with regard to adjacent bases (Fasman, 1975).

Results

Oligonucleotide Products from S-1 Nuclease Digestion. If all duplex oligonucleotide structures suffered from poor base pairing at their termini, S-1 would be expected to function as an exonuclease sequentially removing single nucleotides from both the 3′ and 5′ ends (Drew, 1984). When the dodecamer, $d(CG)_6$, was treated with S-1, hydrolysis proceeded rapidly as if the base pairing at the duplex termini were rather loose. The striking instability of this duplex would certainly argue for an unusual end structure, but simple base-pair fraying alone could not account for the sole formation of $d(CG)_5$ and $d(CG)_4$[1] as the oligonucleotide products. Only fragments that

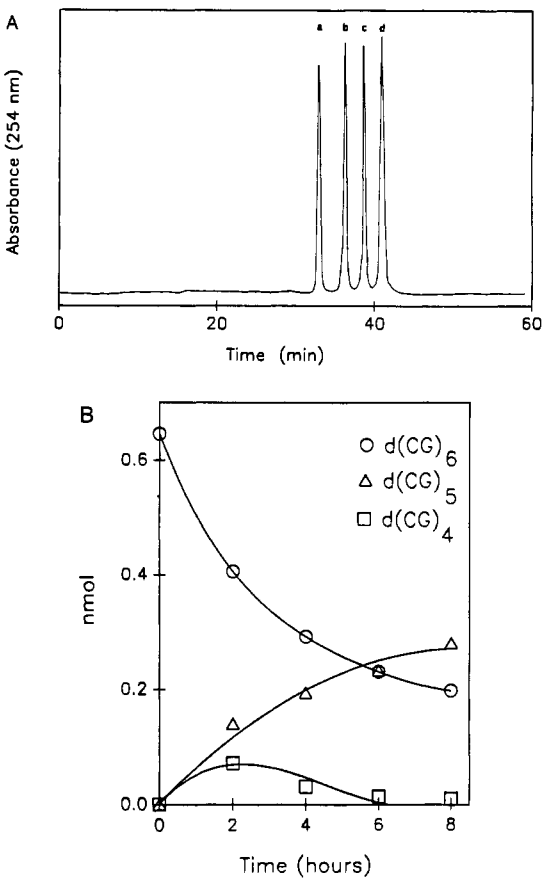


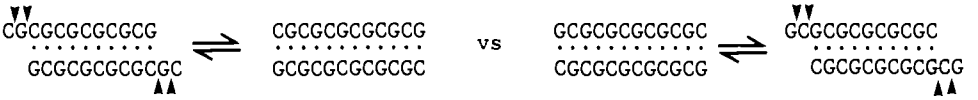
FIGURE 1: (A) Ion exchange chromatography used for analytic and preparative separation of oligonucleotides. DNA fragments [(a) $d(CG)_3$; (b) $d(CG)_4$; (c) $d(CG)_5$; (d) $d(CG)_6$] were separated according to nucleotide length under denaturing conditions on a Mono Q (Pharmacia) anion exchange column with a gradient of 0–825 mM NaCl (50 min, 1 mL/min) in 11.5 mM NaOH. (B) The hydrolysis products of S-1 action on $d(CG)_6$ were followed over time with the described chromatographic separations. An annealed solution of $d(CG)_6$ (5.2 nmol of oligomer) was incubated at room temperature with 10 000 units of S-1 nuclease in 800 μL of 10 mM potassium phosphate, pH 7, and 100 mM NaCl. Aliquots (97 μL) were removed at the indicated times for product analysis as above.

remained in register with the repeating unit of d(CG) were ever detected (Figure 1B). This phenomenon is best explained by the existence of DNA hybrids containing unpaired, overhanging bases that were readily available for S-1 digestion. Duplex structures of $d(CG)_6$ in solution should then be depicted as a series of hybrids (eq 1) that produce unpaired bases two (per strand) at a time.

After the first 2 h of incubation (Figure 1B), approximately 40% of $d(CG)_6$ was consumed to yield $d(CG)_5$ and $d(CG)_4$. The molar quantity of these shortened oligomers represents greater than 90% of the digested starting material and indicates that the primary products of enzyme digestion were the decamer and octamer. A concerted process that would have

[1] S-1 is known to cleave single-strand regions of DNA specifically and yield 5′-phosphorylated termini (Ando, 1966), but the chromatographic analysis used to quantify products in this study was unable to distinguish between phosphorylated and nonphosphorylated oligonucleotides 9–12 bases in length. Therefore, the designation of an enzyme product such as $d(CG)_5$ is not meant to denote the chemical state of the terminal base but rather to describe the size of the oligonucleotide only. Differentiation of the phosphorylated compounds began to emerge for smaller DNA fragments, and these were then characterized after treatment with alkaline phosphatase. For consistency and simplicity, terminal phosphorylation is not generally indicated in this paper, and its presence or absence would not affect any of the important conclusions drawn from the described experiments.

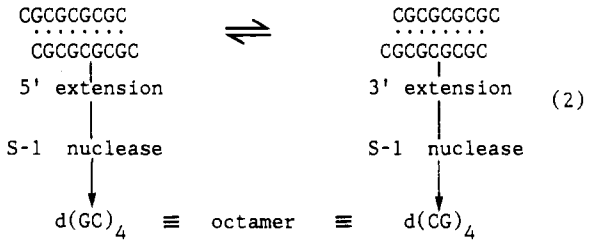
Scheme I: Polarity of DNA Sequence versus Site of S-1 Digestion



completely degraded a minor population of $d(CG)_6$ to its nucleotide components could not have occurred and therefore cannot be invoked to explain the hypersensitivity of $d(CG)_6$. This latter type of conversion would have been easily detected by the formation of a disproportionately high concentration of nucleotides at the expense of oligomer products.

The low molecular weight fragments that were detected after enzyme turnover coeluted with dC, dpC, and dpG under denaturing ion exchange chromatography; no dCG was evident. The mononucleoside and mononucleotides most likely resulted from the sequential digestion of each unpaired base at the 3′ and 5′ termini. A primary release of dCG (or pdCG) followed by a secondary hydrolysis of the dinucleotide to dC (pdC) and pdG was unlikely since this potential intermediate was not quickly degraded by S-1. Under conditions equivalent to those described in Table I, the half-life of this dinucleotide was 4.8 h, almost 50% of that for $d(CG)_6$.

Integrity of Enzyme Specificity. Of course, structural characterization of redundant sequences would be seriously impaired if S-1 were found to deviate from a strict and singular specificity for unpaired DNA. The absolute specificity of S-1 (Vogt, 1973; Drew, 1984) was confirmed here by challenging the enzyme with a series of related oligomers including $d(CG)_4C$ and $d(CG)_3C$ that could not form fully paired structures nor variants with a two-base overhang (eq 2).



(2)

Hybridization of these would only allow for duplexes containing an odd number of unpaired bases and a minimum extension of one base. Enzyme modification of these structures yielded initial fragments (Figure 2), octamer and hexamer, respectively, dictated only by the single-base extension.[2] Further reaction of the nascent octamer remained consistent with that monitored independently with homogeneous samples of $d(CG)_4$. In each case, hydrolysis occurred only at sites adjacent to the smallest repeating unit from which a duplex structure could be formed.

Still, arguments could arise suggesting that an artifact of S-1 specificity instead of facile strand slippage generated the hyperreactivity described for the series $d(CG)_n$. An enzymatic preference for reaction at sites of 5′-G↓C- rather than at sites of 5′-C↓G- would also predict the formation of only $d(CG)_{n-2}$ products. This is not a likely occurrence since the overall degradation described above appeared to proceed stepwise with hydrolysis occurring at both sites. However, lack of a neighboring base effect on enzymatic processing required confirmation through direct experimentation. This was best demonstrated by studying a redundant sequence of opposite polarity, $d(GC)_6$, that contained sites of 5′-C↓G- between the repeating dinucleotide instead of sites of 5′-G↓C- as above (Scheme I). S-1-catatlyzed hydrolysis of $d(GC)_6$ proceeded much like that of $d(CG)_6$. The stable products detected were also those expected from an equilibrium that allowed for strand slippage by two base pairs (Figure 3) and could not have resulted from any pattern of absolute sequence recognition dictated by the enzyme. Degradation was then still regulated by the availability of single-strand regions of DNA even when rather short oligonucleotides were used.

Kinetics of DNA Modification by S-1 Nuclease. The initial and sustained rates during S-1-catalyzed degradation of DNA further illustrated the exceptional solution properties of the redundant sequences. For kinetic measurements, oligonucleotide consumption was again detected discontinuously by ion exchange chromatography, and in every case, the loss of parent DNA followed a first-order, exponential decay. Under standard conditions (Table I), $d(CG)_6$ was converted

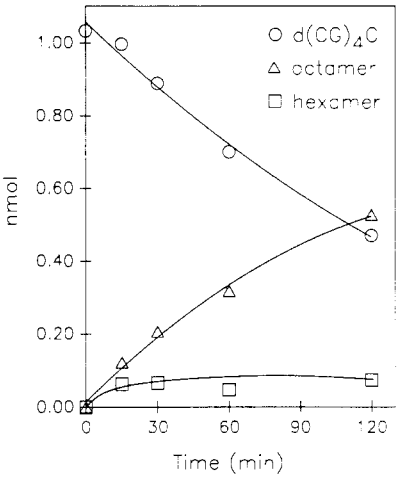


FIGURE 2: Hydrolysis products of $d(CG)_4C$ during digestion with S-1. An annealed solution of $d(CG)_4C$ (6.2 nmol) was incubated at room temperature with 3000 units of S-1 nuclease in 600 μL of 10 mM potassium phosphate, pH 7, and 100 mM NaCl. Aliquots (97 μL) were removed at the indicated times for product analysis as in Figure 1.

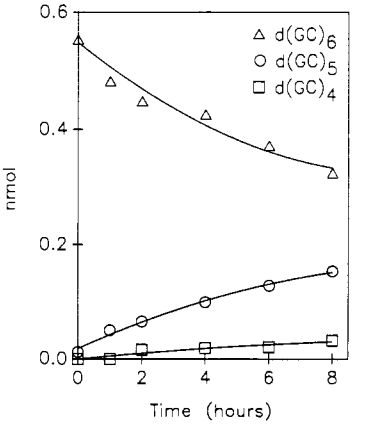


FIGURE 3: Hydrolysis products of $d(GC)_6$ during digestion with S-1. An annealed solution of $d(GC)_6$ (3.9 nmol) was incubated at room temperature with 3500 units of S-1 nuclease in 700 μL of 10 mM potassium phosphate, pH 7, and 100 mM NaCl. Aliquots (97 μL) were removed at the indicated times for product analysis as in Figure 1.

[2] $d(CG)_n$ also coeluted with $d(GC)_n$, and as indicated in eq 2, either $d(CG)_4$ or $d(GC)_4$ could be produced by S-1-dependent hydrolysis of 3′ or 5′ displaced structures, respectively. A possible bias toward preferential formation of one of these structures is currently under investigation.

Table I: Thermal Stability of Duplex Oligonucleotides versus Rate of Degradation in the Presence of S-1 Nuclease[a]

duplex	$T_m \pm 1$ (°C)	$T_{1/2}$ in the presence of S-1 nuclease ± 10% (h)
5′-d(CACGGGTGCGCATG) 3′-d(GTGCCCACGCGTAC)	61	>60
5′-d(CGCGCGCGCGCG) 3′-d(GCGCGCGCGCGC)	>80	11
5′-d(CGCGCGCGCG) 3′-d(GCGCGCGCGC)	71	9.8
5′-d(CGCGCGCGC) 3′-d(CGCGCGCGC)	63	0.90
5′-d(CGCGCGCG) 3′-d(GCGCGCGC)	60	9.9
5′-d(CGCGCGC) 3′-d(CGCGCGC)	50	0.35
5′-d(CGCGCG) 3′-d(GCGCGC)	<50	0.33

[a] Optical melting temperatures were monitored by the change of absorbance at 260 nm vs T (°C) for each duplex structure under the same conditions as for nuclease digestion. The T_m values were determined at $(1/2)(\Delta A_{260})$ (Cantor & Schimmel, 1980). S-1-dependent hydrolysis of each duplex was measured at constant oligonucleotide strand concentrations of ca. 6 μM under conditions similar to those of Figures 1–3, and the loss of starting structure fits well to a first-order exponential decay. Rates are expressed as average ($N \geq 2$) half-life values per 5000 units/mL S-1, and experimental errors are less than ±10%.

to $d(CG)_5$ and $d(CG)_4$ quite readily. In contrast, a duplex of heterogeneous sequence that was unable to form overhanging termini without significant loss of base pairing was an extremely poor substrate for S-1 modification. Only after extended incubation in the presence of a high concentration of enzyme could degradation of this duplex be detected (Table I). Facile degradation is therefore not common to all duplex oligomers but instead is unique to redundant sequences such as $d(CG)_6$.

Conformations of the DNA with blunt and annealed ends were not likely recognized by S-1, and only those oligonucleotides that could stabilize dangling end structures were actively processed. Oligonucleotides such as $d(CG)_6$, $d(CG)_5$, and $d(CG)_4$ reacted at a similar rate with S-1 (Table I), suggesting that each sequence had an equivalent propensity for undergoing strand slippage and subsequent recognition by S-1. The degradation rates for these sequences did not represent a saturation or maximum of enzyme activity, however. Oligonucleotides that were incapable of full complementation, $d(CG)_4C$ and $d(CG)_3C$, reacted over an order of magnitude faster than sequences that could hybridize completely.

The rate of $d(CG)_6$ consumption increased linearly with both enzyme and DNA concentration over the range of 1500–10000 units/mL S-1 and 2–20 μM oligonucleotide. Analytical use of this nuclease for determining solution conformation of DNA was therefore a second-order process; neither enzyme nor active substrate was present in excess. Every attempt to saturate the degradation process failed. Even when such enzyme activators as Zn^{2+} or Mg^{2+} were added to the incubations, DNA digestion only continued to increase. Homogeneous reaction of $d(CG)_6$ was detected whether complete degradation required less than 3 h or greater than 24 h; distinct hybrid forms of DNA and their rate of interconversion could not be distinguished kinetically under these experiments.

Stability and Multiplicity of Hybridized Structures. Thermal transitions of DNA (T_m) were measured optically under conditions parallel to those of the S-1 incubations (Table I). Such analyses were used in this context only to determine the relative stability of oligonucleotide hybridization with respect to the bulk process of a helix to coil transition. This comparison would not necessarily indicate the dynamic formation of intermediates along the melting process. The duplex conformation maintained by the heterogeneous sequences required a lower temperature to fully denature all base pairs than that necessary for the shorter duplex of $d(CG)_6$. Yet, the seemingly less-stable duplex persisted much longer than any other form of DNA in the presence of S-1 nuclease since no alternative base-pairing arrangements of this duplex were stable under ambient conditions. In contrast, the redundant sequences readily formed a variety of structures that did not require complete loss of complementation and would generate suitable targets for S-1 digestion.

Absorbance measurements over a wide range of temperatures also confirmed another trait of symmetric oligonucleotides, a second and more subtle transition at approximately 30–40 °C (data not shown). This characteristic has previously been assigned to an interconversion between duplex and hairpin species and is not likely due to slipped structures (Scheffler et al., 1968; Xodo et al., 1988a, 1989). Hence, the extended helixes proposed in eq 1 are not the only unusual structures that could form with redundant sequences. However, product and kinetic studies implicated enzymatic recognition of only DNA conformations containing dangling ends and not those forming a hairpin loop. As described earlier, DNA fragments associated with S-1 digestion of such a loop were never detected; conversion of $d(CG)_6$ led predominantly to $d(CG)_5$ and $d(CG)_4$ rather than $d(CG)_3$ or an excess of dC, pdC, and pdG. Additionally, $d(CG)_3T_4(CG)_3$, a control oligonucleotide that remained as a hairpin even under ambient conditions (Ikuta et al., 1986), was studied in parallel with the experiments summarized in Table I. The half-life of this DNA was 1.9- and 46-fold greater than that for $d(CG)_6$ and $d(CG)_3$, respectively, in the presence of S-1 and demonstrated that a hairpin along with other complicating structures (Mishra et al., 1988) should not be invoked to explain the unusual reactivity of $d(CG)_6$.

Discussion

Alternative Structures for Duplex $d(CG)_6$. Complementary strands of DNA in vitro and in vivo are most often expected to adopt a helical form that maximizes the standard base-pairing arrangements. Although few would argue with this general principle, a significant number of sequences have now been shown to hybridize into a variety of alternative conformations (Wells, 1988; Barton, 1988). Many of these exceptional complexes arise from symmetric or redundant sequences within DNA (François et al., 1988; Henderson et al., 1987; Oka & Thomas, 1987; Sen & Gilbert, 1988; Sheardy & Swinkle, 1989; Xodo et al., 1988b). The model system presented here illustrated that the overall reactivity of DNA can be influenced and, in this case, even controlled by the multiplicity of available solution structures. The specificity of S-1 revealed the facility by which $d(CG)_6$ maintained a population of helixes containing unpaired and extended termini even under ambient conditions and physiological pH.

Both product identification and kinetic measurement of oligonucleotide turnover with S-1 could be correctly predicted and understood through a mechanism of strand slippage from within hybridized structures. Sequences that displayed a hyperreactivity were those that maintained strong interactions with their complement while still managing to equilibrate from a species containing blunt ends to one with extended ends. All

oligonucleotide products detected after nuclease digestion indicated that base pairing for all conformations remained in register with the repeating unit of the copolymer (Figures 1B, 2, and 3).

Throughout these studies, S-1 reacted with an unerring specificity for unpaired and accessible nucleotides. The anomalous degradation of copolymeric DNA could not have been controlled by a hitherto unknown preference for certain sequences. Hydrolysis was catalyzed at sites of 5′-C↓G- and 5′-G↓C- in both $d(CG)_6$ and $d(GC)_6$. The possibility that a redundant sequence such as $d(CG)_6$ may form a non-B, non-Z conformation cannot be directly addressed in this study (Suggs & Wagner, 1986; Fowler & Skinner, 1986; Evans & Efstratiadis, 1986). However, this is not required to explain the action of S-1 on these oligonucleotides. Hairpin forms of sequences such as $d(CG)_3N_2(CG)_3$ have also been identified (Xodo et al., 1988a) but equivalent structures formed by oligonucleotides studied here are similarly insufficient to account for this hyperreactivity. In fact, the description of enzymatic specificity above is prefaced with the adjective "accessible" to accurately portray the enzyme's weak reaction with the unpaired bases of a small loop region. No hydrolysis at the thymidine residues of $d(CG)_3T_4(CG)_3$ was detected under the described conditions, and this was quite consistent with the slow digestion of another loop region noted previously (Drew, 1984).

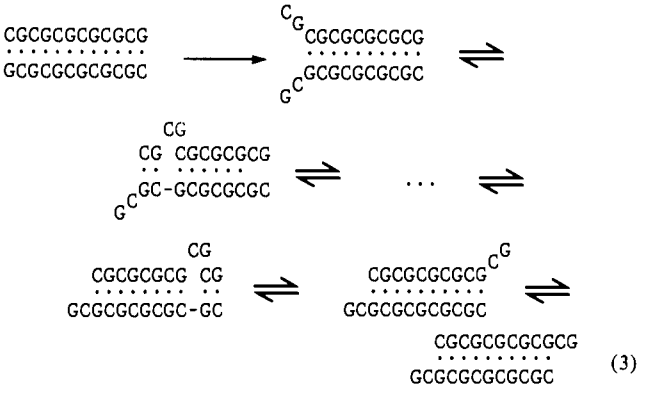
Dynamics and Solution Equilibria of $d(CG)_n$. Neither the relative rate of $d(CG)_6$ hydrolysis nor the extent of degradation was consistent with the traditional, static model for DNA hybridization. If base pairs were formed solely during the annealing process, at least two discrete populations of $d(CG)_6$ would result, one quite reactive to S-1 and the other very inert. Yet, such a distinction was not evident; degradation of $d(CG)_6$ proceeded at an intermediate and linear first-order rate consuming at least 75% of the dodecamer over the course of a typical 24-h incubation (Table I). Furthermore, the rate was found directly dependent on both DNA and enzyme concentrations. The unusual lability of this sequence seems then to be a general characteristic of the entire population and not unique to a specific fraction of DNA. Sequences such as $d(CG)_4C$ that were unable to fully base pair reacted with this enzyme over an order of magnitude faster than $d(CG)_6$. All forms of $d(CG)_4C$ in solution fulfilled the requirement for a substrate of S-1 whereas only the misaligned and least-stable forms of $d(CG)_6$ would qualify as substrates. A comparison of the rates indicated here may therefore reflect the relative concentration of DNA available for enzymatic reaction.

Melting temperatures (T_m) associated with DNA hybridization are an undisputed measure of the net stability of duplex formation, yet a correlation between the bulk properties of a duplex and the chemical reactivity of its individual solution structures is not so apparent. The conformation of the major reacting species may differ greatly from the time-average conformation measured by thermal denaturation. By comparison of T_m values (Table I), the 14-base heterogeneous duplex would be considered less stable than the dodecamer $d(CG)_6$. By S-1 hydrolysis, the converse was true. Even among the series $d(CG)_6$, $d(CG)_5$, and $d(CG)_4$, T_m values did not accurately predict the relative rates of S-1-induced degradation. The solution properties of $d(CG)_6$, $d(CG)_5$, and $d(CG)_4$ are distinguishable by T_m, but all react similarly with S-1. Very rapid hydrolysis is not evident until $n = 3$ for $d(CG)_n$.

The sensitivity of the S-1 activity with the dodecamer, decamer, and octamer must result from the sequences' common propensity to form an equilibrium of structures. The overall stability of hybridization may decrease for shorter oligomers, but the approximate fraction of misaligned structures should remain constant. Assuming every C-G base pair in the duplex provides three hydrogen bonds, the first shifted structure of $d(CG)_6$, $d(CG)_5$, or $d(CG)_4$ represents the same loss of six hydrogen bonds. The fractional loss, however, increases for the smaller oligomers, growing from 17% for $d(CG)_6$ to 25% for $d(CG)_4$. Before an exact ratio of aligned vs misaligned duplexes can be accurately predicted, other features that stabilize duplex structures must be more fully described. For example, oligonucleotide hybridization has already been found to strengthen in the presence of dangling ends (Freier et al., 1985; Senior et al., 1988), and an overall stability is certainly influenced by the polarity of base stacking (Manzini et al., 1987).

Mechanism for Strand Slippage. Various proposals might explain the homogeneous reactivity of $d(CG)_6$ including a counterintuitive idea that a substantial fraction of the dodecamer does not fully hybridize in solution. Alternately, a transition state of low relative energy may lie between the fully annealed complex and misaligned hybrids, allowing for equilibration between structures even under mild conditions. In this case, a constant small percentage of misaligned conformers would be available for enzyme hydrolysis under conditions of a second-order reaction, just as observed. Finally, an S-1-induced melting of local structure prior to hydrolysis cannot be absolutely dismissed, yet this explanation is unlikely since $d(CG)_6$ was also found to be hyperreactive under other conditions such as exonuclease III digestion (Rokita and Romero-Fredes, unpublished results).

A facile equilibration between hybridized structures (eq 1) is then most reasonable if a mechanism can avoid full denaturation during the migration of base pairs. Otherwise, a highly energetic process would be required to match the high temperature necessary to melt the hybridized forms of $d(CG)_6$. A general unraveling of duplex termini cannot determine S-1 susceptibility since duplexes of heterogeneous sequence seem not particularly susceptible to S-1 digestion. However, this unraveling may initiate a low-energy isomerization to form the reactive misaligned complex characteristic of redundant sequences (eq 3). If two base pairs temporarily open and then



(3)

re-form hydrogen bonds to the adjacent set of nucleotides, a bulge would form transiently. This could reverse itself or migrate through the hybrid until two unpaired termini are produced (Scheffler et al., 1968). The illustrated intermediates of strand displacement relinquish hydrogen bonding between only two base pairs, the same loss as that sustained in the product. The transitory structures that would allow for migration of the bulge (not shown) require no more than two additional base pairs to separate momentarily.

Previous studies have demonstrated that a small bulge can migrate through redundant sequences quite quickly (Woodson & Crothers, 1987, 1988). In the present investigation, this rapid equilibrium was demonstrated by a heightened and homogeneous reaction beween $d(CG)_6$ and S-1 that was likely dominated by only a minor conformation of DNA. A similar flexibility in the structure of cellular DNA must then adversely affect the accuracy of sequence complementation and may therefore describe a molecular basis for the origin of frameshift mutations in vivo [for example, see de Boer and Ripley (1988) and Kunkel and Soni (1988)].

ACKNOWLEDGMENTS

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Registry No. d(CACGGGTGCGCATG)·d(CATGCGCACCCGTG), 123639-54-3; d(CGCGCGCGCGCG), 92950-48-6; d(CGCGCGCGCG), 114742-74-4; d(CGCGCGCGC), 123411-34-7; d(CGCGCGCG), 89991-79-7; d(CGCGCGC), 112079-75-1; d(CGCGCG), 58927-26-7; S-1 nuclease, 37288-25-8.

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