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Action of Single-Strand Specific Nucleases on Model DNA Heteroduplexes of Defined Size and Sequence†

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ABSTRACT: The sensitivity of the model DNAs containing dA·dG and dG·dG heteroduplex regions of defined length to S_1 and mung bean single-strand specific nucleases was tested by polyacrylamide gel electrophoretic analysis of the distribution of product oligonucleotides. Single-base mismatch heteroduplexes were extremely resistant to these nucleases, although low levels of cleavage at the heteroduplex nucleotide were observed at high nuclease concentrations. The nuclease sensitivity of dA·dG heteroduplex regions increased gradually as the length of the heteroduplex region increased from one to six nucleotides. The sensitivity of dG·dG heteroduplexes three to five nucleotides long was considerably greater than that of the single dG·dG mismatch.

The reason for our interest in determining the susceptibility of regions of nonpaired nucleotides to single-strand specific nucleases was presented in the preceding paper of this issue (Dodgson and Wells, 1977). Also, the preparation, characterization of, and t_M studies on defined heteroduplex DNAs were reported.

In other studies, single-strand specific nucleases have been used to probe for other types of irregular structural sites in double-stranded DNA. First, these nucleases specifically cleave some superhelical DNAs, perhaps due to transient or permanent unwinding of the helix in order to decrease the superhelical density (Beard et al., 1973; Wiegand et al., 1975; Germond et al., 1974; Kato et al., 1973; Mitra et al., 1976; Wang, 1974). These nuclease-sensitive sites may either be in specific regions of the superhelical DNA (Beard et al., 1973; Germond et al., 1974) or may occur at random throughout the molecule (Wiegand et al., 1975; Mitra et al., 1976; Bartok and Denhardt, 1976), depending on the type of DNA studied. Second, these nucleases specifically cleave heteroduplex DNAs containing insertion loops at the mutant site (Wiegand et al., 1975; Shenk et al., 1975); it has been proposed that the nucleases can, in certain cases, cleave heteroduplex molecules at sites of single-base change mutations (Shenk et al., 1975; Legerski et al., 1976). Third, S_1 nuclease cuts nicked DNA in the strand across from the break (Beard et al., 1973; Wiegand et al., 1975; Germond et al., 1974; Shenk et al., 1975; Shishido and Ando, 1975). It also weakly cleaves nucleotides (or small oligomers) from the ends of DNA duplexes (Shenk et al., 1975), an action termed "nibbling". Fourth, mung bean nuclease preferentially cleaves double-stranded DNA at regions containing high percentages of dA·dT base pairs (Chan et al., 1977; Johnson and Laskowski, 1970). While the single-strand specificity of these nucleases has suggested that sensitive sites in duplex DNA molecules must exist at least transiently as single-strand loops or bubbles, the actual site of recognition on duplex DNA is unclear, since many of the sensitive sites are of unknown structure.

On the basis of the S_1 and mung bean nuclease susceptibility studies on biosynthetic model DNAs with heteroduplex regions of defined length, we conclude that single-base change heteroduplexes can be cleaved only with extremely low efficiency, and that nuclease sensitivity increases substantially as heteroduplex size increases above three nucleotides in length.

Materials and Methods

Enzymes. S_1 nuclease was purified as described (Vogt, 1973) with the omission of the sulfo-Sephadex chromatography. Units are as described by Vogt, although in some cases the units were measured by the hydrolysis of radioactive λ DNA in the presence of calf thymus DNA carrier at 37 °C and converted to Vogt units by a previously determined correction factor. The specific activity of the S_1 nuclease was 120 000 units/mg of protein. Mung bean nuclease was purified as described (Chan, 1976) and was the generous gift of H. W. Chan.

Other Materials. Nucleotides, nucleic acids, and other materials were described previously (Dodgson and Wells, 1977).

Synthesis of Defined Heteroduplex and Corresponding Homoduplex DNAs. All materials and methods pertaining

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to the synthesis and characterization of these molecules were described in the preceding paper in this issue (Dodgson and Wells, 1977).

Nuclease Reaction Mixtures. Standard S_1 nuclease reactions were as follows: approximately 0.3 A_{250} unit of heteroduplex DNA was incubated at about 1.0 A_{250} unit/mL in 0.03 M sodium acetate buffer, pH 4.6, 0.05 M NaCl, 1.0–1.5 mM $ZnSO_4$, and 5% glycerol. The amount of S_1 nuclease varied as described. Incubations were for 30 min at 37 °C. Reactions were terminated with addition of EDTA[1] (to 8 mM) and Tris base (to 15 mM). Oligo(deoxynucleotides) were liberated from $(dG)_n$ by the addition of excess $(dC)_n$ (about 5 mol/mol of $(dG)_n$), followed by heating to 95 °C for 3 min and rapid cooling on ice.

Mung bean nuclease incubations were in 0.03 M ammonium acetate buffer, pH 5.0, 1 mM $ZnSO_4$ at 37 °C for 30 min and were terminated as described above.

Gel Electrophoresis. Reaction mixtures were made 5 to 15% in sucrose, and about 0.04 A_{270} unit of $(dC)_8$ or $(dC)_9$ marker oligonucleotide was added. Samples (up to 0.5 mL in volume) were electrophoresed on 20% polyacrylamide gels as described previously (Burd and Wells, 1974, detailed methods of gel preparation and electrophoresis are available on request from the authors). Electrophoresis was at 2.5 mA/gel for about 3 h or until a bromophenol blue marker ran 6 to 9 cm into the gel. The gels were scanned for absorbance at 270 nm in a Gilford 2410 gel scanning system. A background level of 0.2 A_{270} unit was subtracted from all gel scans shown. As previously demonstrated (Burd and Wells, 1974), this system will separate individual oligo(deoxynucleotides) (e.g., $(dC)_m$ from $(dC)_{m+1}$) up to about 40 nucleotides in length (Figure 1A). The logarithm of the length (or molecular weight) of a homologous oligomer series gives a linear plot of negative slope when graphed vs. electrophoretic mobility (i.e., log m vs. R_f for $(dC)_m$). R_f values relative to the standard marker oligonucleotides can thus be converted to oligomer length when compared to a standard curve determined from gel electrophoresis of a pancreatic DNase digest of $(dC)_n$.

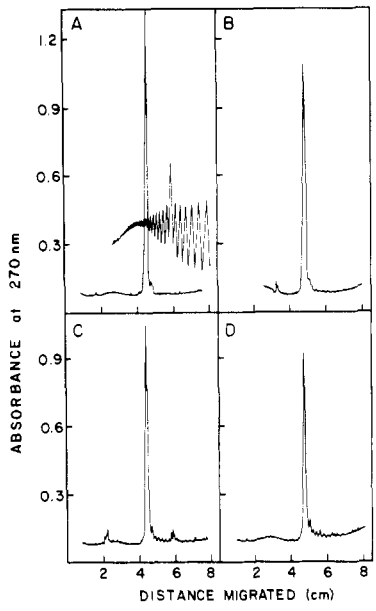

FIGURE 1: The action of S_1 nuclease on the homoduplex $(dG)_n$·$(dC)_{27}$. $(dG)_n$·$(dC)_{27}$ (0.24 A_{250} units) was incubated with 0 (A), 20 (B), 100 (C), and 400 (D) units of S_1 nuclease in standard 0.1-mL reaction mixtures (Methods) for 30 min at 37 °C. The resultant oligomers were liberated from $(dG)_n$ by competition with 0.68 A_{270} unit of $(dC)_n$ and electrophoresed on 20% polyacrylamide gels from left to right. The major peak in each panel has a mobility corresponding to that of $(dC)_{27}$. The upper curve in panel A shows the absorbance scan of a parallel gel on which 0.5 A_{270} unit of a DNase digest of $(dC)_n$ (Dodgson and Wells, 1977) was electrophoresed. About 0.01 A_{270} unit of $(dC)_{19}$ marker was added to the digest. Adjacent peaks differ in size by one nucleotide; thus, the enhanced $(dC)_{19}$ peak allows identification of all other $(dC)_m$ peaks that are resolved. This scan was positioned by alignment of the $(dC)_{27}$ peaks of the two curves in panel A.

Results

S_1 Nuclease Action on $(dG)_n$·$(dC)_{27}$. As a control for the studies of S_1 nuclease action on heteroduplex regions surrounded by dG·dC base pairs, we first studied the action on the corresponding homoduplex $(dG)_n$·$(dC)_{27}$. The homoduplex (Dodgson and Wells, 1977) was incubated with various levels of S_1 nuclease and the resultant dC oligonucleotides were electrophoresed on 20% polyacrylamide gels (Figure 1). The $(dG)_n$·$(dC)_{27}$ incubated in the absence of enzyme shows low levels of adjacent oligomers (the small faster-migrating peak has a mobility corresponding to that of $(dC)_{25}$). Note that the size of the substrate oligomer peak decreases on incubation with increasing amounts of enzyme. At the highest enzyme level (400 units of S_1 nuclease), about 70% of the $(dC)_{27}$ remains intact. Note that this amount of enzyme is enough to degrade about 400 times as much (100 A_{260} units) heat-denatured λ DNA to acid-soluble fragments; hence, $(dG)_n$·$(dC)_{27}$ is relatively resistant to S_1 cleavage. The degradation results in low levels of product oligonucleotides that run faster than $(dC)_{27}$. Peaks at least as small as $(dC)_{20}$ are clearly visible after treatment by the highest S_1 nuclease level.

No visible product oligonucleotide was seen in the $(dC)_{13}$ area, suggesting that little or no cleavage was initiated at the center of the $(dC)_{27}$ oligomer annealed to $(dG)_n$. Rather, it appears that all cleavage is initiated at the free ends of the oligomer, i.e., the so-called "nibbling" reaction (Shenk et al., 1975). These experiments do not distinguish cleavage at the 3′ end from that at the 5′ end nor do they determine whether only mononucleotides are released by nibbling or if small oligomers (2–4 nucleotides long) are also released.

Results similar to those described above have also been obtained with the homoduplexes $(dG)_n$·$(dC)_{30}$, $(dG)_n$·$(dC)_{22}$, and $(dG)_n$·$(dC)_{15}$. In general, nibbling seems to proceed a little more rapidly as m decreases in the series $(dG)_n$·$(dC)_m$ (results not shown).

S_1 Nuclease Action on $(dG)_n$·$d(C_{12}A_5C\overline{29})$. Polyacrylamide gel electrophoresis is routinely used to analyze the results of nuclease action on the heteroduplex DNAs, since it resolves both the intact substrate and various product oligomers. At high levels of S_1 nuclease, even the homoduplex $(dG)_n$·$(dC)_{27}$ is weakly attacked; hence, a decrease in the amount of intact substrate heteroduplex oligomer is not sufficient to indicate cleavage at the heteroduplex region. Rather, we have generally taken the appearance of product oligomers corresponding to the blocks of dC residues to the 5′ and 3′ side of the heteroduplex region as a condition for attack at the heteroduplex site. Since the dC block on the 5′ end of the oligomer is defined in length (i.e., $(dC)_{12}$), this product oligomer is most easily seen on the gels. The 3′ block of dC residues is distributed over a broad enough range in many cases that not a sufficient amount of any single product length is produced to be quantitated accurately. Also, these product oligomers might be mistaken for products of nibbling on the substrate oligomers. (Such product

[1] Abbreviations and nomenclature were described in the adjoining paper (Dodgson and Wells, 1977).

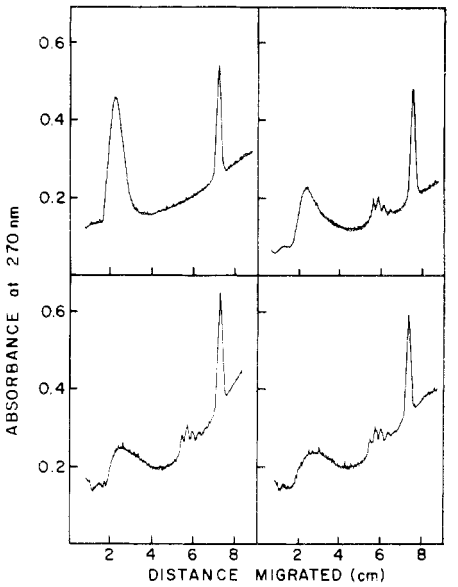

FIGURE 2. The action of S_1 nuclease on $(dG)_n$·$d(C_{12}A_5C_{\overline{29}})$. The heteroduplex DNA was incubated under standard S_1 nuclease reaction conditions (0.34 A_{250} unit in 0.34 mL) with 0 (upper left), 34 (upper right), 85 (lower left), and 850 (lower right) units of S_1 nuclease. Incubations were for 30 min at 37 °C and were terminated as described (Methods). 0.95 A_{270} unit of $(dC)_n$ was added and the mixture was boiled for 3 min and cooled on ice. After the addition of sucrose and 0.04 A_{270} unit of $(dC)_9$, the solution was electrophoresed on 20% polyacrylamide gels and the gels were scanned. Electrophoresis is from left to right; the sharp peak on the right side of each panel is the $(dC)_9$ marker oligonucleotide.

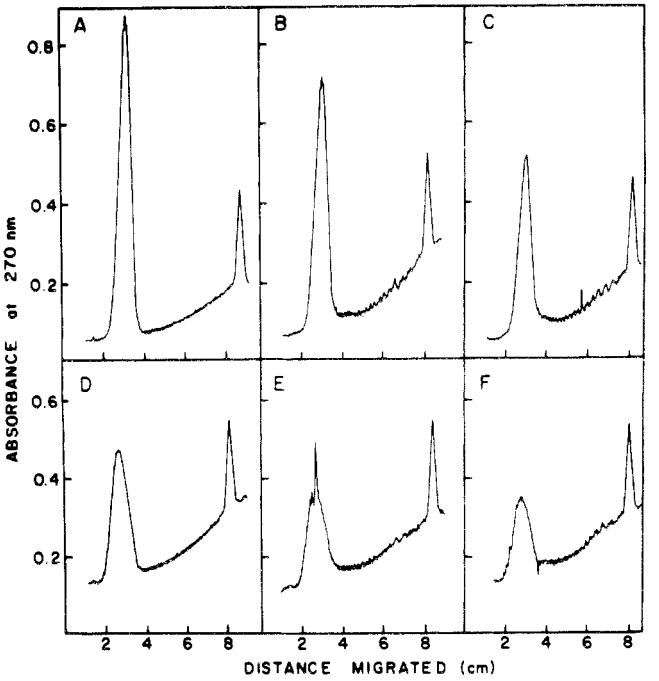

FIGURE 3: The action of S_1 nuclease on $(dG)_n$·$d(C_{12}AC_{\overline{25}})$ and on $(dG)_n$·$d(C_{12}A_2C_{\overline{28}})$. 0.62 A_{250} unit of $(dG)_n$·$d(C_{12}AC_{\overline{25}})$ (A–C) and 0.45 A_{250} unit of $(dG)_n$·$d(C_{12}A_2C_{\overline{28}})$ (D–F) were incubated under standard reaction conditions (0.34 mL) with 0 (A and D), 85 (B and E), 850 (C and F) units of S_1 nuclease for 30 min at 37 °C. Reactions were terminated and prepared for gel electrophoresis as described (Methods) with the addition of $(dC)_9$ marker oligonucleotide. Electrophoresis is from left to right; the sharp peak at the right of each panel is the $(dC)_9$ marker.

oligomers may appear as a rapidly migrating shoulder on the intact oligomer peak.)

S_1 nuclease cleavage of a dA·dG heteroduplex five nucleotides long is illustrated in Figure 2. The oligonucleotide electrophoresis profiles of untreated $(dG)_n$·$d(C_{12}A_5C_{\overline{29}})$ and those of the heteroduplex treated with three levels of S_1 nuclease are shown. The oligomer $(dC)_9$ is added to each electrophoresis sample and appears at the right of each scan. S_1 nuclease treatment of these heteroduplexes results in the appearance of oligomer peaks migrating just behind the $(dC)_9$ marker. These peaks have mobilities corresponding to $(dC)_{12}$, $d(C_{12}A)$, $(dC_{12}A_2)$, and $d(C_{12}A_3)$ from right to left. $(d(C_{12}A_4)$ and $d(C_{12}A_5)$ might be produced by cleavage of the heteroduplex and the terminal dA residues rapidly trimmed by the S_1 nibbling reaction such that only $d(C_{12}A_3)$ and smaller oligomers appear on the gel.) Note that, while increasing enzyme concentrations decrease the amount of intact substrate oligomer observed (peak at left of the scan), very little increase in the product $d(C_{12}A_{0-3})$ peak is seen. This is apparently due to the more rapid rate of degradation by S_1 nuclease of small dC oligomer products relative to that of longer substrate oligomers. Note also that the cleavage product oligomers that arise from the blocks of dC residues to the 3′ side of the heteroduplex region are not resolved but form a broad shoulder on the more rapidly migrating side of the intact substrate oligomer peak.

S_1 Nuclease Action on $(dG)_n$·$d(C_{12}AC_{\overline{25}})$ and $(dG)_n$·$d(C_{12}A_2C_{\overline{28}})$. As mentioned in a preliminary report of this work (Dodgson, 1976), early attempts to cleave dA·dG heteroduplexes one or two base oppositions in length by the method used above were unsuccessful. That is, no cleavage product oligonucleotide peaks were visible above background on the 20% analytical gels. Recent improvements in our scanning system (use of a Gilford 250 rather than a 240) and the electrophoresis of greater amounts of treated heteroduplex DNA have now allowed the detection of lower levels of product oligonucleotide (Figure 3).

In this figure, the sensitivities of $(dG)_n$·$d(C_{12}AC_{\overline{25}})$ and $(dG)_n$·$d(C_{12}A_2C_{\overline{28}})$ to moderate and high levels of S_1 nuclease are shown. Again, the $(dC)_9$ marker oligonucleotide peak is at the right of each panel and the substrate oligonucleotide peak on the left. Low levels of product oligonucleotide peaks are seen just behind the $(dC)_9$ peak in gel runs of both the S_1-treated heteroduplexes. These peaks indicate cleavage of the DNAs at the heteroduplex region, but their small area suggests only about 5% of the substrate oligomer is cleaved. This is a minimum estimate, however, since some of the product oligonucleotide is lost due to the nibbling reaction. Note that the decreases in the uncleaved substrate oligomer peaks in panels C and F are substantial ($\simeq$50%), but, as described above, part of this decrease is due to nibbling rather than cleavage at the heteroduplex region. Indeed, products of the nibbling reaction (along with product oligonucleotides from the 3′ end of the cleaved heteroduplex molecules) can be seen slightly above background between the uncleaved substrate oligomer and the main product oligomers. The major product oligonucleotide peaks have mobilities corresponding to those of $(dC)_{11}$, $(dC)_{12}$, $d(C_{12}A)$. These results indicate that the one and two nucleotide heteroduplexes are sensitive to S_1 nuclease cleavage, but they react at a relatively lower rate than does the five nucleotide heteroduplex (see above).

S_1 Nuclease Action on $(dG)_n$·$d(C_{12}A_{3-6}C_{\overline{30}})$. A comparison of the action of a moderately high level of S_1 nuclease on dA·dG heteroduplexes 3, 4, 5, and 6 bases in length is shown in Figure 4. After nuclease treatment, the amount of product oligonucleotide running just behind the $(dC)_9$ marker increases with increasing heteroduplex size from 3 to 5 bases in length. Also the amount of intact substrate oligomer after S_1 treatment

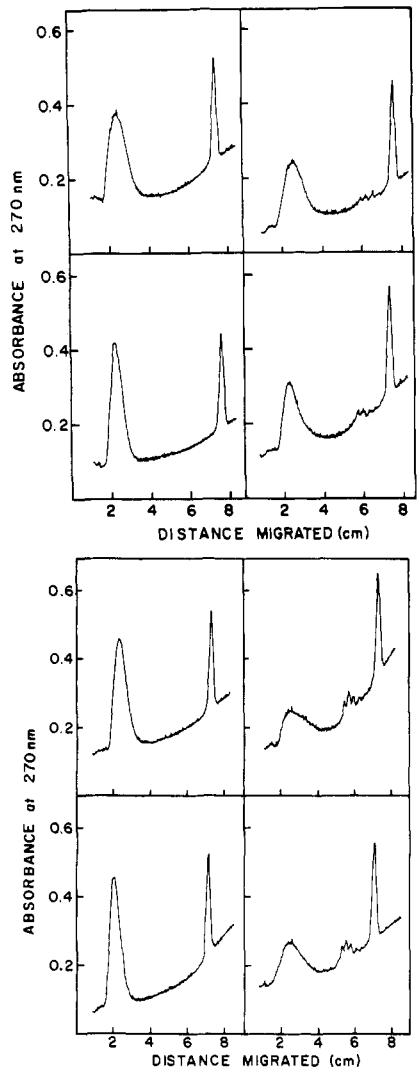

FIGURE 4: The action of S_1 nuclease on heteroduplexes of the form $(dG)_n$·$d(C_{12}A_mC_{\bar{x}})$, where m = 3 (top, A), 4 (bottom, A), 5 (top, B), and 6 (bottom, B). In all cases, $\bar{x} = 30 \pm 1$. The heteroduplex DNA preparations (0.32 A_{250} unit in 0.34 mL) were incubated under standard S_1 nuclease conditions (Methods) in the absence of nuclease (left) or in the presence of 85 units of nuclease (right) for 30 min at 37 °C. Reactions were terminated and prepared for electrophoresis as described (Methods) with addition of $(dC)_9$ reference oligonucleotide. Electrophoresis on 20% gels is from left to right. The sharp peak on the right side of each panel is the marker $(dC)_9$.

TABLE I: Acid-Insoluble Radioactivity Remaining After Treatment of $(dG)_n$·$d(C_{12}A_mC_{\bar{x}})$[a] with S_1 Nuclease.

S_1 Nuclease (units/mL)	% [^{3}H]dA Remaining Acid Insoluble[b]					
	$m = 1$	$m = 2$	$m = 3$	$m = 4$	$m = 5$	$m = 6$
50	95	96	Nd[c]	Nd[c]	79	73
250	95	78	73	57	55	63
500	85	81	77	69	51	42

[a] Substrate heteroduplexes synthesized as described were of the form $(dG)_n$·$d(C_{12}[^3H]A_mC_{\bar{x}})$, where $m = 1, \bar{x} = 25$; $m = 2, \bar{x} = 28$; $m = 3, \bar{x} = 29$; $m = 4, \bar{x} = 31$; $m = 5, \bar{x} = 29$; and $m = 6, \bar{x} = 31$. [b] Standard S_1 nuclease reactions (Methods) were incubated at 37 °C. Five percent trichloroacetic acid precipitable ^{3}H cpm was measured at 0 and 30 min. 100% = 300 to 1000 cpm. The concentrations of substrate heteroduplexes (in A_{250} units/mL) were 1.8 and 1.2 for m = 1 and m = 2, respectively, and were 0.95 for m = 3-6. [c] Nd, not determined.

is about 70, 60, 40, and 40% of the untreated oligomer for heteroduplexes 3, 4, 5, and 6 bases in length, respectively.

This relative order of sensitivity to S_1 nuclease is also observed in a comparison of the rates of nuclease conversion of radioactive label in the heteroduplex dA nucleotides to an acid-soluble form (Table I). The ^{3}H radioactivity remaining acid-insoluble increases as the length of the dA heteroduplex block decreases. These results, along with those of Figure 4, indicate that the dA·dG heteroduplexes become increasingly sensitive to S_1 nuclease as their size increases from one to at least five bases long.

The acid-insolubility results are complicated by the fact that both cleavage at the heteroduplex region and nibbling of the exposed dA nucleotides are necessary for the [^{3}H]dA label to become completely acid soluble. Oligomers less than about 20 nucleotides in length are, however, partially acid soluble (Cleaver and Boyer, 1972). Therefore, part of the decrease in acid-insoluble radioactivity may also be due to the nibbling of oligomers which are uncleaved at the heteroduplex region. The amount of labeled heteroduplex remaining acid insoluble after nuclease treatment can, however, be used as a relative measure of its insensitivity to cleavage at the heteroduplex region and can be correlated to the results of the gel analyses (i.e., compare Table I, line 2, to Figure 4).

S_1 Nuclease Action on $(dG)_n$·$d(C_{10}G_{3-5}C_{\bar{x}})$. A variety of heteroduplex DNAs containing defined lengths of dG·dG mismatched bases was also synthesized. The S_1 nuclease sensitivity of these heteroduplexes closely resembles that of the dG·dA heteroduplexes described above. The action of two levels of S_1 nuclease on heteroduplexes of the form $(dG)_n$·$d(C_{10}G_mC_{\bar{x}})$, where m = 3, 4, and 5, is shown in Figure 5. This figure shows incubation in the absence of S_1 nuclease (left) or in the presence of two levels of the enzyme. The oligomer $(dC)_8$ was added as a marker and is seen at the right of each panel. S_1 nuclease cleavage results in the appearance of three product oligonucleotide peaks migrating just behind the $(dC)_8$ marker. The mobilities of these three peaks correspond to those of $(dC)_{10}$, $d(C_{10}G)$, and $d(C_{10}G_{3-5})$ from right to left ($d(C_{10}G_2)$ probably migrates with the $d(C_{10}G_{3-5})$ peak). These peaks therefore correspond to the oligomers from the 5′ side of the cleaved heteroduplex DNAs.

The oligomers from the 3′ side of the cleaved region ($\bar{x}$ = 23, 20, and 17 from top to bottom, respectively) run as a distribution between the intact substrate oligomer peak and the major product oligomer peak. Note that similar amounts of cleavage by these high nuclease levels are observed whether the heteroduplex is three, four, or five bases long. These three heteroduplexes also show similar sensitivities to S_1 nuclease at lower enzyme levels (results not shown).

S_1 Nuclease Action on $(dG)_n$·$d(C_{10}GC_{\overline{25}})$. The sensitivity of a single base dG·dG mismatch to S_1 nuclease was studied. The reaction on $(dG)_n$·$d(C_{10}GC_{\overline{25}})$ with up to 835 units of enzyme was performed and analyzed under standard conditions. As discussed (Dodgson and Wells, 1977), the preparation of $(dG)_n$·$d(C_{10}GC_{\overline{25}})$ contained about 25% $(dG)_n$·$d(C_{10}G_{3-5}C_{\overline{22}})$. Less cleavage by S_1 nuclease is observed with this heteroduplex sample than was seen in the three to five base heteroduplex samples (data not shown). This is the case whether cleavage is measured by the decrease in the intact substrate oligomer peak or by the amount of product oligomer. Due to the presence in the sample of the three to five base heteroduplex molecules, it is not possible to determine whether any of the product oligomer results from cleavage of the sin-

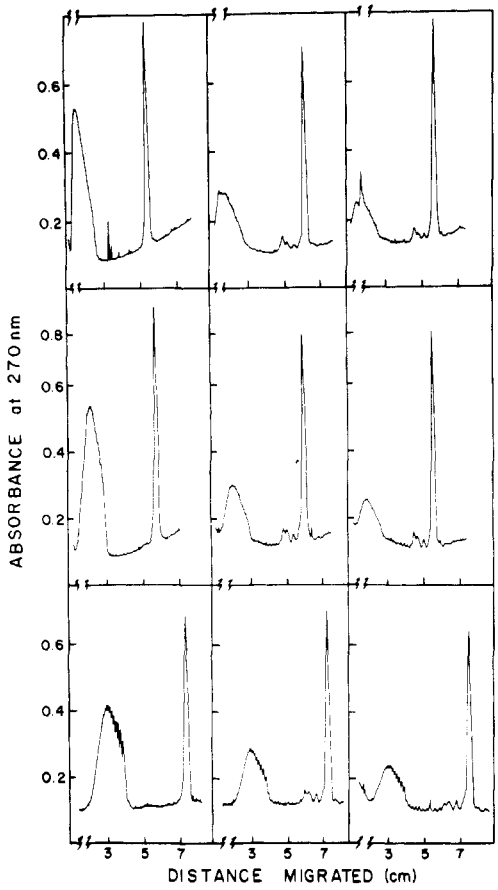

FIGURE 5: The action of S_1 nuclease on heteroduplexes of the form $(dG)_n$·$d(C_{10}G_mC_{\bar{x}})$, where m = 5 (top), 4 (center), and 3 (bottom) and $\bar{x}$ = 23, 20, and 17, respectively. The heteroduplexes prepared as described were incubated under standard conditions (0.48 A_{250} unit in 0.325 mL) for 30 min at 37 °C and terminated as described (Methods). Reactions contained 0 (left), 325 (middle), or 1000 (right) units of S_1 nuclease. Oligomers were freed from $(dG)_n$ and electrophoresed as described (Methods) after the addition of $(dC)_8$ marker oligonucleotide. Electrophoresis is from left to right. The sharp peak on the right side of each panel is the $(dC)_8$ marker.

gle-base heteroduplex. The presence of a product oligomer peak with a mobility corresponding to that of $d(C_{10}G_{3-5})$ (and possibly $d(C_{10}G_2)$) suggests that much of the product oligomer distribution results from S_1 action on the three to five base heteroduplex contaminant. Thus, it appears that, as in the dA·dG heteroduplex case, the single-base mismatch is cleaved only weakly, if at all, by S_1 nuclease.

Discussion

Nuclease Sensitivity of Heteroduplex Regions. The S_1 nuclease susceptibility of defined heteroduplex oligomer·polymer complexes was described. The dA·dG and the dG·dG single-base mismatch heteroduplexes were quite resistant to S_1 nuclease, although low levels of cleavage were observed. Sensitivity to cleavage increased gradually as the dA heteroduplex block complexes with $(dG)_n$ increased in length from one to six nucleotides. Heteroduplexes containing blocks of dG nucleotides three to five nucleotides in length complexed to $(dG)_n$ all had about the same sensitivity to S_1 nuclease, but were much more sensitive than the single dG·dG mismatch. These results were confirmed with studies on a variety of heteroduplexes of the form $(dG)_n$·$d(C_{12}A_mC_{\bar{x}})$ and $(dG)_n$·$d(C_{10}G_mC_{\bar{x}})$ (Dodgson and Wells, 1977) in which $\bar{x}$ differed from that in the experiments described above. Variations in $\bar{x}$ resulted in only slight differences in nuclease susceptibility, as assayed by both acid insolubility and gel electrophoresis (results not shown).

It should be emphasized that the S_1 nuclease levels tested on these heteroduplex DNAs varied from about 10 to 1000 times the amount necessary for complete degradation of an equal amount of single-stranded DNA (heat-denatured calf thymus or λ DNA). Lower enzyme levels showed little or no observable cleavage of the heteroduplex DNAs. Therefore, these complexes are rather resistant to the nuclease in comparison with single-stranded nucleic acids or even in comparison with a duplex DNA of low thermal stability, such as $(dA)_n$·$(dT)_n$, which is approximately 10% as sensitive as single-stranded calf thymus DNA (unpublished results).

The sensitivities of the heteroduplex complexes to mung bean nuclease also were examined. At all nuclease levels tested, similar results to those described above were obtained (data not shown). The heteroduplex sensitivity to mung bean nuclease relative to that of denatured λ DNA was, within a factor of two, equal to their sensitivity to S_1. (Concentrations of mung bean nuclease above 100 times that necessary to degrade an equal amount of denatured λ DNA could not be tested on heteroduplexes.)

Variations in the zinc ion concentration (0.5–4.5 mM), NaCl concentration (50–300 mM), and incubation temperature (30–56 °C) also had no major effect on the cleavage of the heteroduplex DNAs by S_1 nuclease (results not shown). Slight variations in heteroduplex sensitivity could be explained solely by changes in enzyme activity, as measured on heat-denatured calf thymus DNA rather than by any effect on the substrate DNA. At 56 °C, however, only low levels of product oligomer peaks were observable, presumably due to the instability of the complexes of such small oligomers (approximately $(dC)_{12}$) with $(dG)_n$ in the S_1 reaction mixture.

Certain reservations regarding the extrapolation of the results from these model polymer heteroduplex experiments to heteropolymeric DNA studies must be mentioned. First, we have examined heteroduplexes involving only two of the eight possible noncomplementary base oppositions. Whereas the results were rather similar for the dA·dG and dG·dG oppositions, the nuclease susceptibility of other heteroduplex types may differ. For example, dG·dT heteroduplexes may form structures that are even less nuclease susceptible due to the well-documented dG·dT base pairing possibilities (Gillam et al., 1975).

Secondly, we cannot be sure of the influence of the highly thermostable $(dG)_n$·$(dC)_m$ blocks on the structure of the heteroduplex region. The stabilizing effect of blocks of dG·dC base pairs on adjacent regions of DNA was demonstrated previously (Burd et al., 1975b) and, in the case of the model heteroduplexes, these blocks may inhibit the formation of heteroduplex loops. Unfortunately, the nuclease susceptibility of $(dA)_n$·$(dT)_n$ precludes the study of heteroduplex DNA regions bordered by dA·dT blocks.

Also it should be noted that the heteroduplex bases in the model polymers may not be held in a single stable structure (i.e., loop, bubble, or stacked region) due to the possibility of migration of the dC blocks along the $(dG)_n$ chain. Such migration of adjoining duplex DNA regions would not easily occur in heteropolymer DNAs.

S_1 Nuclease as a Probe for DNA Structure. With the aforementioned reservations in mind, our results are relevant to the interpretation of several previous studies employing single-strand specific nucleases as structural probes. First, the observation of a uniquely nuclease-sensitive site near the lac-

tose operator in λ p*lac* DNA led to three models (Chan and Wells, 1974) for the unusual structural site (thermolabile blocks of DNA, hairpin loop, and base-paired DNA of non-DNA B structure). If the site contains nonpaired nucleotides, our results suggest that several contiguous unpaired nucleotides would need to be involved in a region of such nuclease sensitivity. Furthermore, our results suggest that the nuclease-sensitive sites in supercoiled DNAs (see Introduction) involve several unpaired bases. It is not certain if these could be transiently unpaired as suggested previously (Wang, 1974) or may be stably unpaired as favored by other workers (Woodworth-Gutai and Lebowitz, 1976).

Finally, it would seem unlikely that single-stranded specific nucleases would be useful in the isolation of a block of DNA between two single-base mismatch heteroduplexes with reasonable yield. Recently, such an experiment has been tested (Wells et al., 1977) using heteroduplex DNA fragments between wild-type lactose promotor DNA (*E. coli*) and DNA of either single base mismatch or single-base deletion mutants of known nucleotide sequence (Dickson et al., 1975). No cleavage products of the fragment by S_1 or mung bean nuclease were observed on analytical polyacrylamide gels. The sensitivity of this experiment was such that the low level of S_1-nuclease cleavage of putative single-base change heteroduplexes observed previously (Shenk et al., 1975) might not have been visible (approximately 10% cleavage of the heteroduplex fragments would be necessary for detection). However, these results along with the model polymer studies certainly suggest that S_1 nuclease will not be generally useful in the isolation of DNA segments bordered by two single mismatch heteroduplex sites.

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