Differential Effect of Hg(II) on $[d(A)_n \cdot d(T)_n]$ and $[d(A\text{-}T)_n \cdot d(A\text{-}T)_n]$ Sequences: Circular Dichroism (CD) Measurements and Endonuclease Digestion Studies Using Poly[d(A)·d(T)] and Poly[d(A-T)·d(A-T)] as Substrates

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The long-wavelength positive CD bands of poly[d(A)·d(T)] and poly[d(A-T)·d(A-T)] become inverted upon the addition of $Hg(ClO_4)_2$. Poly[d(A)·d(T)] requires higher levels of mercury to undergo inversion than poly[d(A-T)·d(A-T)]. Mercurated poly[d(A)·d(T)] is digested more rapidly than the control by DNase I or staphylococcal nuclease at low levels of $Hg(ClO_4)_2$. Let $r \equiv [Hg(ClO_4)_2]_{added}/[DNA\text{-}P]$. A 4- to 5-fold rate increase occurs with DNase I at $r = 0.25$; a 2-fold increase with staphylococcal nuclease at $r = 0.2$. By contrast, digestion of poly[d(A-T)·d(A-T)] decreases immediately with increasing r. The noted rate increases appear to be due to a modification of poly[d(A)·d(T)] helix structure prior to the chiroptical conversion. The modification is interpreted as a widening of the minor groove, permitting, thus, a better binding of DNase I to its substrate. The overall changes in CD as well as enzymatic digestion rates are taken to signal mercury-induced alterations in helix screwness from right-to-left. They are totally reversible subsequent to the removal of mercury.

Introduction

As shown in previous communications [1–4], $Hg(ClO_4)_2$ (denoted Hg(II)) induces conformational alterations in B-form nucleic acids that, on the basis of the accompanying changes in their circular dichroism (CD), support the notion of transitions taking place from the right-handed form to one with a left-handed screw sense. The chiroptical changes were observed with calf thymus DNA [1, 3] as well as with the synthetic nucleic acids poly[d(A-T)·d(A-T)], poly[d(G-C)·d(G-C)] [2], and poly[d(T-G)·d(C-A)] [4]. It was further found that the reversible inversion of the long-wavelength Cotton band of mercurated calf thymus DNA (maximum at 273 nm) from positive to negative chirality is accompanied by an equally reversible decrease in the rate of digestion of the DNA by staphylococcal nuclease [3]. The enzyme is known not to digest left-handed DNA [5].

In this communication, we report on the differential effect of Hg(II) on the conformation of poly[d(A-T)·d(A-T)] and poly[d(A)·d(T)] as monitored *via* circular dichroism measurements and enzymatic digestion studies, employing the two endonucleases DNase I and staphylococcal nuclease. DNase I was additionally selected since it, too, does not digest left-handed DNA [5].

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Materials and Methods

Poly[d(A)·d(T)], poly[d(A-T)·d(A-T)], and DNase I (bovine pancreas, EC 3.1.21.1, lot 70H9605, activity 2000 units/mg of protein) were purchased from Sigma. Staphylococcal nuclease, Foggi strain (EC 3.1.31.1, lot 4798), was a product of Worthington. Its activity was 17,278 units/mg of protein. Information regarding the commercial suppliers as well as analytical properties of the other chemicals used in this work can be found elsewhere [1–4].

Circular dichroism measurements were performed at 25 °C as described [1–4]. The polymers, dissolved in 0.1 M $NaClO_4$, 5 mM cacodylic acid buffer, pH 6.9, were at a final concentration near 0.84 A^{260}-units (about 42 µg/ml). Polymer concentrations were evaluated by using the molar absorptivities of 6000 and 6650 (l/mol(P)/cm) at 260 nm for poly[d(A)·d(T)] [6] and poly[d(A-T)·d(A-T)] [7], respectively. In the case of poly[d(A-T)·d(A-T)], $Hg(ClO_4)_2$ was always added to a fresh sample; this kept volume dilution well below 1% and volume corrections were not necessary. To

save material, poly[d(A)·d(T)] was titrated with $Hg(ClO_4)_2$. The volume dilution, amounting maximally to 3.2%, was taken into consideration when calculating molar circular dichroism $\Delta\epsilon \equiv \epsilon_L - \epsilon_R$. ϵ_L and ϵ_R are, as usual, the molar absorptivities of left-handed and right-handed circularly polarized light. H g(II) levels are given as $r \equiv ([Hg(ClO_4)_2]_{added}/[DNA\text{-}P])$. The brackets represent molar concentrations.

The methodology of the enzymatic digestion experiments has been described in detail elsewhere [3]. DNase I hydrolysis rates were determined in 0.1 M $NaClO_4$, 5 mM cacodylic acid buffer, pH 6.9 (37 °C); staphylococcal nuclease rates were measured in 0.1 M $NaClO_4$, 5 mM boric acid buffer, pH 8.9 (37 °C). Final component concentrations in the salt solvents amounted to: polymers, 50 µg/ml; DNase I, 100 units/ml with poly[d(A)·d(T)] and 10 units/ml with poly[d(A-T)·d(A-T)] (in presence of 4.2 mM $Mg(ClO_4)_2$); staphylococcal nuclease, 1–2 units/ml for either polymer (in presence of 2 mM $Ca(ClO_4)_2$). Absolute rates of enzymatic digestion ($R_{(r=0)}$) are given in terms of A^{260} units of released oligonucleotides min^{-1} $unit^{-1}$ of enzyme. They were found to be as follows (rates of the control $r = 0$): staphylococcal nuclease (poly[d(A)·d(T)], $R_{(r=0)} \cdot 10^4 = 270.3$; poly[d(A-T)·d(A-T)], $R_{(r=0)} \cdot 10^4 = 559.8$); DNase I (poly[d(A)·d(T)], $R_{(r=0)} \cdot 10^4 = 0.63$; poly[d(A-T)·d(A-T)], $R_{(r=0)} \cdot 10^4 = 23.8$). Relative digestion rates are given by $R_{rel} \equiv R_{(r>0)}/R_{(r=0)}$.

Results

Circular dichroism measurements

The CD spectra of the Hg(II) complexes of the two synthetic nucleic acids are displayed in Fig. 1 (poly[d(A)·d(T)] and 2 (poly[d(A-T)·d(A-T)]). Although of identical chemical overall composition, the polynucleotides differ in base sequence and, hence, also in their stacking interactions. Consequently, their spectra bear no resemblance to one another. The spectrum of untreated poly[d(A)·d(T)] ($r = 0$) consists of three positive and three negative Cotton effects in the wavelength range 360–200 nm: going in the direction of shorter wavelengths, the maxima of the three positive bands are located at 281 ($\Delta\epsilon_{max} = +1.925$), 258 ($\Delta\epsilon_{max} = +3.377$), and 216 nm ($\Delta\epsilon_{max} = +9.388$). The three negative bands are at 267

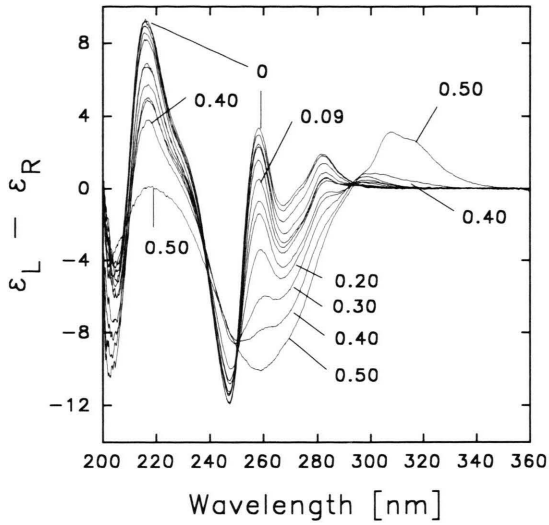

Fig. 1. Circular dichroism spectra of poly[d(A)·d(T)] in presence of $Hg(ClO_4)_2$ in 0.1 M $NaClO_4$, 5 mM cacodylic acid buffer, pH 6.9. The numbers with the curves are r-values whereby $r \equiv [Hg(ClO_4)_2]_{added}/[DNA\text{-}P]$. The brackets refer to molar concentration. To avoid overcrowding of the figure, not all r-values are shown. However, their progression (sequence: 0; 0.01; 0.03; 0.05; 0.07; 0.09; 0.12; 0.15; 0.20; 0.30; 0.40; 0.50) becomes obvious when perusing the changes of the 258 nm band. Spectra were recorded at 25 °C in a 1 cm pathlength cuvette. All spectra are solvent as well as cuvette corrected. For further details, consult text.

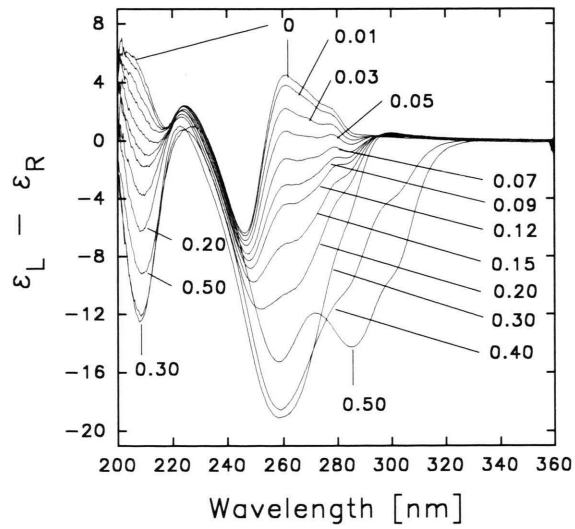

Fig. 2. Circular dichroism spectra of poly[d(A-T)·d(A-T)] in presence of $Hg(ClO_4)_2$ in 0.1 M $NaClO_4$, 5 mM cacodylic acid buffer, pH 6.9. The numbers with the curves are r-values. For further information, consult the legend of Fig. 1 as well as text.

($\Delta\epsilon_{max}$ = −0.933), 247 ($\Delta\epsilon_{max}$ = −11.390), and 205 nm ($\Delta\epsilon_{max}$ = −4.247). Positive and negative bands are separated from one another by cross-over points at 273, 264, 254, 234, 209, and 200 nm.

A brief report on the mercury-induced CD changes of poly[d(A-T)·d(A-T)] has already appeared [2]. The polymer was reinvestigated, however, so as to have a common basis with the poly[d(A)·d(T)] data. The CD spectrum of untreated poly[d(A-T)·d(A-T)] (r = 0) is somewhat more complex than that of poly[d(A)·d(T)]: at first glance, there are only two positive and one negative Cotton effects: a major positive band at 261 nm ($\Delta\epsilon_{max}$ = +4.501) and a minor positive band at 224 nm ($\Delta\epsilon_{max}$ = +2.436). The negative band has its extreme value at 246 nm ($\Delta\epsilon_{max}$ = −6.346). It will be noted, however, that the 261 nm band has a shoulder at 277 nm ($\Delta\epsilon_{max}$ = +2.309) and that there is a "trough" at 217 nm in the region of positive chirality. Lastly, the shoulder stretching towards 200 nm belongs to a strong positive band at 194 nm with $\Delta\epsilon_{max}$ = +15.010 (not shown). Positive and negative bands are separated by the cross-over points at 253 and 233 nm.

Fig. 1 and 2 also show that each polynucleotide responds differently to Hg(II). Since mercury levels were raised in each case in the sequence r = 0; 0.01; 0.03; 0.05; 0.07; 0.09; 0.12; 0.15; 0.20; 0.30; 0.40; 0.50, the spectra can be compared with one another. Higher r-levels (*e.g.*, r = 0.75, 1.00, and 2.00) have been omitted from the figures because neither DNase I nor staphylococcal nuclease digest the associated complexes *(vide infra)*. Also, the corresponding CD spectra do not differ much from the r = 0.5 spectra.

Both poly[d(A)·d(T)] and poly[d(A-T)·d(A-T)] see their long-wavelength positive CD bands ultimately converted to bands of large negative chirality. However, higher levels of Hg(II) are needed with the former than with the latter to bring about inversion. Thus, the 281 and 258 nm bands of poly[d(A)·d(T)] are "pushed" below the zero CD line at $r \geq 0.12$ and loose their "positive" appearance at $r \geq 0.30$ while with poly[d(A-T)·d(A-T)] equivalent shifts occur at $r \geq 0.05$ and $r \geq 0.15$, respectively (261 nm band and 277 nm shoulder). Perusal of Fig. 1 reveals that the following isodichroic points are generated during the addition of Hg(II): two come into being at 240 and 250 nm from r = 0 to r = 0.12 and a third exists in the range $0 \leq r \leq 0.5$ at 295 nm; the two isodichroic points at 240 and 250 nm vanish at $r > 0.12$ and are replaced by a point at 245 nm in the range $0.3 \leq r \leq 0.5$. For poly[d(A-T)·d(A-T)] (Fig. 2), the following relations hold: isodichroic points exist at 294 and 220 nm up to $r \leq 0.3$; they disappear thereafter and three new ones form at 275, 225, and 215 in the range $0.3 \leq r \leq 0.5$. The fact that both poly[d(A)·d(T)] and poly[d(A-T)·d(A-T)] possess more than one set of isodichroic points when titrated with Hg(II) shows that there are minimally two equilibria operative: (1) right-handed ↔ modified right-handed and (2) modified right-handed ↔ left-handed. Additional points of interest are the following: (1) poly[d(A)·d(T)], at r = 0.5, develops a positive band at 308 nm with $\Delta\epsilon_{max}$ = +3.135 and a shoulder at 317 nm with $\Delta\epsilon_{max}$ = +2.651, while poly[d(A-T)·d(A-T)], at the same r-level, does not. In fact, the spectrum of the latter enters here a region of negative chirality; (2) the negative CD signals of poly[d(A)·d(T)] increase in strength from, say, $\Delta\epsilon^{205} \approx -4.1$ (r = 0) to $\Delta\epsilon^{205} \approx -8.3$ (r = 0.3), while the signals of poly[d(A-T)·d(A-T)] progress from $\Delta\epsilon^{205} \approx +5.7$ (r = 0) to $\Delta\epsilon^{205} \approx -10.8$ (r = 0.3), *i.e.*, undergo inversion. Curiously enough, mercury, at r = 0.5, tends to shift the signals back to more "positive" values: thus, $\Delta\epsilon^{205} \approx -3.2$ with poly[d(A)·d(T)] and $\Delta\epsilon^{205} \approx -7.5$ in the case of poly[d(A-T)·d(A-T)]; (3) the negative 247 nm band of poly[d(A)·d(T)] is quite invariant with Hg(II) in the range $0 \leq r \leq 0.2$ (although it merges at $r \geq 0.3$ with the inverted 258 nm signal), while the negative 246 nm CD signal of poly[d(A-T)·d(A-T)] deepens and is increasingly red-shifted with increasing mercury levels.

Finally, all CD changes are completely reversible upon the removal of Hg(II) from the polymers. This can be done by adding NaCN (up to a 10-fold molar excess) or KI (up to a 4-fold molar excess; higher KI levels tend to distort the spectra at low wavelengths). The CD reversibility demonstrates that Hg(II), upon binding to DNA, keeps all bases in register. This, incidentally, holds whether the DNA is a mixed sequence polymer (*e.g.*, calf thymus DNA) or consists of non-random, regular sequences (*e.g.*, the substances used here).

Enzymatic studies

In Fig. 3 is shown the variation of the relative rates of digestion (R_{rel}) of poly[d(A)·d(T)] and

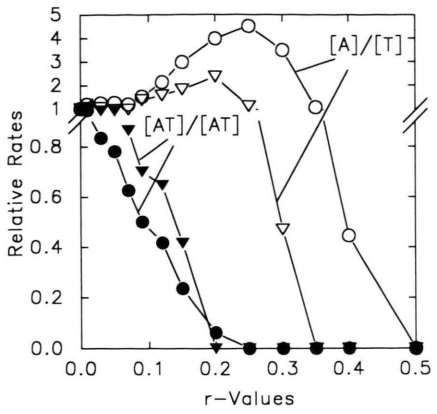

Fig. 3. Relative rates of endonucleolytic digestion of poly[d(A)·d(T)] (open symbols, [A]/[T]) and poly[d(A-T)·d(A-T)] (closed symbols, [AT]/[AT]) by staphylococcal nuclease (triangles) or DNase I (circles) as a function of $Hg(ClO_4)_2$ concentration (r-values). Rate of the control = 1. Staphylococcal nuclease hydrolysis rates were determined at 37 °C in 0.1 M $NaClO_4$, 5 mM boric acid buffer, pH 8.9; DNase I hydrolysis rates at 37 °C in 0.1 M $NaClO_4$, 5 mM cacodylic acid buffer, pH 6.9. Please note that the rates along the rate axis are not uniform. For further details, consult text.

poly[d(A-T)·d(A-T)] by DNase I or staphylococcal nuclease as a function of mercury concentration. Both staphylococcal nuclease and DNase I cease hydrolyzing poly[d(A-T)·d(A-T)] at or near $r = 0.2$; the corresponding situation exists with poly[d(A)·d(T)] at $r = 0.35$ and $r = 0.5$, respectively. In no instance was there enzymatic activity at $r > 0.5$. It is of interest to note that the rate of digestion of poly[d(A)·d(T)] by staphylococcal nuclease increases with increasing mercury concentration in the range $0 \leq r \leq 0.25$, reaching a maximum at $r = 0.2$, and that an even more formidable increase in endonucleolysis is observed with DNase I in the range $0 \leq r \leq 0.35$. A maximum exists here at $r = 0.25$. By contrast, no such rate increases are found with poly[d(A-T)·d(A-T)]: DNase I's rate of digestion declines immediately upon the addition of Hg(II) while the digestion by staphylococcal nuclease remains first unaffected up to $r = 0.05$ and then declines with increasing values of r (*cf.* Fig. 3).

As with CD, the enzymatic rate changes can be reversed by removing Hg(II) with the aid of strong complexing reagents. Both cyanide (up to a 10-fold molar excess) and iodide (up to a 4-fold molar excess) can be employed in the case of staphylococcal nuclease; with DNase I, only iodide can be used since cyanide inhibits irreversibly the enzyme. High levels of KI tend to inhibit the enzyme, too. Rate recovery is complete and yields $R_{r=0}$ (within ±7% or lower).

Discussion

Hg(II) is known to interact strongly and yet reversibly with the nitrogen-binding sites of purines and pyrimidines [8–13]. It is believed that with duplex DNA the metal is chelated between the Watson-Crick base pairs, forming strong bonds to the σ electron pairs of nitrogen atoms in a linear =N–Hg–N= configuration (sp-hybridization) [10, 13, 14]. Strand cross-linking does not appear to disturb the alignment of opposing bases too severely because removal of the mercuric ions with suitable strong complexing reagents fully restores the biological activity of previously mercurated DNA [15].

With free nucleosides, the affinity of mercury to nitrogen decreases in the sequence N3 (T) > N1 (G) ≫ N_{other} (A, C, G) [12]. In polynucleotides, bonding to N7 (or N3) of (G) plays a role, too [16]. Mercury binding to N7 can, in principle, induce Z-form DNA. With mercurated poly[d(G-C)·d(G-C)], the left-handed Z-form is known to exist [2, 16]. The data of this work, as well as the results of previous work [1–4], would indicate that mercury-induced left-handed conformations can also be generated in AT-polymers, or in mixed sequence nucleic acids such as calf thymus DNA. Incidentally, calf thymus DNA is known to convert to the Z-form upon bromination [17].

The changes seen in CD, as well as in the susceptibility to digestion by endonucleases, subsequent to the addition of Hg(II) to poly[d(A)·d(T)] and poly[d(A-T)·d(A-T)] strongly support the notion of conformational alterations occurring in helix structure from right-handed to left-handed screwness:

(1) both staphylococcal nuclease and DNase I do not digest left-handed DNA [5], and endonucleolysis of the two polynucleotides studied here ceases at the points of chiroptical right→left inversion (Fig. 1–3);

(2) right→left transitions are reversible (*e.g.*, B↔Z transitions), and reversible are the CD and enzymatic changes caused by Hg(II);

(3) the fact that the rate of endonucleolysis first increases with increasing r in the case of poly[d(A)·d(T)], but not poly[d(A-T)·d(A-T)], is proof that the observed enzyme effects are substrate-related and have nothing to do with inhibition of the endonucleases. It should be mentioned in this context that nucleic acids, quite in general, have a high affinity for Hg^{2+}. Poly[d(A-T)·d(A-T)], in particular, binds Hg^{2+} so tightly that the concentration of unbound mercury is in the vicinity of $[Hg(II)]_{free} = 10^{-23}-10^{-19}$ M at $0 \leq r \leq 0.5$ [13]. Although r has been defined here in terms of mercury "added" it is, in reality, identical with r_b, the base-equivalent amount of "bound" mercury;

(4) last but not least, from the fact that neither poly[d(A)·d(T)] nor poly[d(A-T)·d(A-T)] absorb (ordinary) electromagnetic radiation at wavelengths above 330 nm at $0 \leq r \leq 0.5$ (not shown) we conclude that mercuration does not generate polynucleotide aggregates. DNA condensates are noted through light scattering in the 700–460 nm range; they are known to be induced in, for instance, calf thymus DNA by trivalent cations such as hexamine cobalt(III) [18, 19]. DNA condensates (*e.g.*, of ψ-type structure) can produce seemingly left-handed conformations although the individual DNA helices in a condensate are believed to remain in the right-handed B-form. A condensed ψ-type structure had been proposed for Hg(II) calf thymus DNA [20] but was rejected by others based on the results of electric dichroism and sedimentation velocity experiments [21] as well as flow linear dichroism measurements [22]. The slight red-shift, then, noted with the polynucleotides upon mercuration (*e.g.*, λ_{max} of poly[d(A)·d(T)] changes from 260 (r = 0) to 274 nm (r = 1.0)) can be explained fully on the basis of the similar red-shifts found with the trimer d(TpTpT) (λ_{max} 266 nm at r = 0 and 272 nm at r = 1.0) or dimer d(ApT) (λ_{max} 262 nm at r = 0 and 274 at r = 1.0) as well as with other dimers and trimers of the AT- and GC-type (Gruenwedel and Cruikshank, publication in preparation). These small molecules are not known to undergo aggregation in the presence of Hg(II). Although there can be little doubt that the CD inversions noted here represent true chiral (right↔left) changes it remains to be seen whether they represent B↔Z transitions or changes between (right-handed)B↔(left-handed)non-B forms.

Why would Hg(II) at (approximately) $0.05 \leq r \leq 0.35$ lead to an increase in endonucleolytic activity with poly[d(A)·d(T)] but not with poly[d(A-T)·d(A-T)]? A definitive answer can be given in the case of DNase I. The enzyme is known to bind to B-form DNA by extending an exposed loop region into the minor groove [23]. Cutting frequency is governed by groove size and helix flexibility. Poly[d(A-T)·d(A-T)] is extremely flexible and is endowed with the normal minor groove width of 12–13 Å [24]. By contrast, poly[d(A)·d(T)] is a very stiff molecule and has a minor groove width of only about 9 Å [25]. An extensive review of its structure ("non-standard B") and solution properties has been presented by Herrera and Chairs [26]. Poly[d(A)·d(T)] is thus a poor substrate for DNase I while poly[d(A-T)·d(A-T)] is an excellent one. This is shown by our finding that $R_{(r=0)}[(AT)\cdot(AT)]/R_{(r=0)}[(A)\cdot(T)] = 37.8$. Hg(II), then, at low levels, must lead to a widening of the minor groove of poly[d(A)·d(T)]; *i.e.*, it induces a better fit between enzyme and substrate, and the rate of digestion increases. It is quite possible that mercuration in the range $0.05 \leq r \leq 0.35$ affects (indirectly) the spine of hydration in the minor groove, changing thereby its conformation from a non-standard B (narrow minor groove) to the standard B form [26]. Since the non-standard B conformation is believed to be stabilized by purine-purine base stacking interactions as well as additional hydrogen bonds, both arising from the high propeller twist of the base pairs [25], the adding on of Hg(II) to the various N-binding sites of the bases could easily alter their stacking interactions as well as hydrogen bonding pattern and, hence, also the base pair propeller twist. In view of the conformational flexibility of the deoxyribose ring it is reasonable that such Hg(II) base interactions should have an immediate effect not only on the structure of the sugar-phosphate backbone but also on its solvation properties. Once the polymer undergoes the right→left inversion, the enzyme-substrate binding weakens and the cutting frequency declines.

Regarding staphylococcal nuclease, the enzyme cuts preferentially A or T at their 5′-sides. A hydrophobic pocket of the enzyme appears to be particularly suited to accept T and A but not C or G [27]. It appears that widening of the minor groove of poly[d(A)·d(T)] by Hg(II) causes its T and/or A

strands to fit better onto the DNA-binding surface of the enzyme, which is a long, narrow cleft [27]. As found in our study, the enzyme's cutting frequency on poly[d(A-T)·d(A-T)] is about twice the one holding for poly[d(A)·d(T)] in absence of mercury. Fig. 3 shows that Hg(II), at $r = 0.2$, causes the two rates to become equal.

One last point. The base sequence of poly[d(A-T)·d(A-T)] is self-complementary, *i.e.*, in addition to interstrand base pairing (formation of perfect duplexes) intrastrand base pairing can also occur. The latter leads to the formation of hairpin helices or loops [28–30]. The question arises what influence, if any, do these structures have on both the CD and the enzymatic digestion of the polymer in the absence as well as presence of Hg(II)? To be sure, Hg(II) should be able to insert itself readily into a loop. If it is assumed that four nucleotides suffice to form a hairpin helix [29], the loop diameter will be approximately 9 Å (taking 7 Å as the intrastrand phosphate–phosphate distance of a B-family chain [31]) and, therefore, large enough to accommodate divalent mercury [32].

We are unaware of work that has looked in a systematic manner at possible effects of hairpin formation or branching on the CD of nucleic acids. However, from the data collected by Greve *et al.* [6], one may deduce that branched or looped structures, if present, are of little consequence to the overall CD. For instance, they compared the CD difference spectra ($CD_{single\text{-}stranded} - CD_{double\text{-}stranded}$) of poly[d(A-T)·d(A-T)], poly[d(A)·d(T)], crab satellite DNA (ranging in base composition from 97–92% AT), and AT-rich *D. melanogaster* satellite DNA to one another and found them to be similar with respect to the wavelength position of their transition extrema. Since the satellite DNAs contain GC base pairs that make chain slippage or hairpin formation unlikely, this similarity appears to rule out intrastrand base pairing as a determining factor in the make-up of the CD of highly polymerized (long-chain) nucleic acids such as poly[d(A-T)·d(A-T)]. The situation may of course be quite different in the case of oligonucleotides with self-complementary short-chain strands.

We believe that poly[d(A-T)·d(A-T)] in 0.1 M $NaClO_4$, 5 mM cacodylic acid buffer, pH 6.9, has few, if any, looped structures. We infer this from the following: the polymer's helix-to-random coil transition midpoint temperature, measured spectrophotometrically with ordinary UV, is at T_m = 60.1 °C and the slope $(d\theta/dT)_{Tm}$ of the denaturation curve amounts to about 0.3/°C. θ is the fraction of hydrogen-bonded bases (Lopez and Gruenwedel, unpublished observation). Almost identical values are obtained when the same polymer is heat-denatured in 47.5 mM Na_2SO_4, 5 mM cacodylic acid, pH 6.8 (M–Na ≈ 0.1); to wit: T_m = 58.8 °C and $(d\theta/dT_m)_{Tm}$ = 0.37/°C [33]. Most importantly, however, when heat denaturation is followed calorimetrically in the 47.5 mM Na_2SO_4 solvent (M–Na ≈ 0.1), employing an adiabatic twin calorimeter, the transition curve does not contain a "premelting" zone. It resembles, in appearance, the curve measured, for instance, in 0.013 m (molal) Na_2SO_4 (see Fig. 1 of ref. [33]). This curve is void of a "premelting" region and it shows an absorbed energy distribution curve that is highly Gaussian. Definite "premelting", however, is noted with poly[d(A-T)·d(A-T)] in 0.907 m (molal) Na_2SO_4 (see Fig. 2 in ref. [33]). Poly[d(A-T)·d(A-T)] is thought to engage in looping and branching at high salt levels [28]. Incidentally, the average helix length L_h (cooperative length) of the polymer in the 47.5 mM Na_2SO_4 medium and at T_m is 42 base pairs, with a nucleation parameter of $\sigma = 5.70 \cdot 10^{-4}$. Herrera and Chairs [26] quote 30 base pairs for the length of the cooperative unit of poly[d(A-T)·d(A-T)] at T_m, which is in good agreement with our findings. Thus, we are quite certain that poly[d(A-T)·d(A-T)] in buffered 0.1 M $NaClO_4$ has no or only marginal levels of highly branched hairpin structures.

Although there exists a vast amount of literature concerning the action of DNase I and staphylococcal (micrococcal) nuclease on poly[d(A-T)·d(A-T)] as well as AT-rich oligonucleotides (*cf.* [34]) no studies seem to have been undertaken that have looked specifically at loop structure effects on the rate of digestion. In view of the fact that oligonucleotides with self-complementary short-chain strands tend to undergo extensive branching, a determination of the cutting frequency and cutting specificity of the two endonucleases on the mercury complexes of such oligonucleotides would be highly desirable.

In conclusion, based on the results obtained in this study, we predict that long stretches of [d(A)$_n$·d(T)$_n$], containing, on the average, one

bound mercury every three-to-five base pairs, are hydrolyzed by endonucleases such as DNase I or staphylococcal nuclease at rates very much larger than the ones to be expected for $[d(A\text{-}T)_n \cdot d(A\text{-}T)_n]$ sequences at the same degree of mercuration. The biological consequences of this are unclear at present although it should be pointed out that $[d(A)_n \cdot d(T)_n]$ oligonucleotide tracts are involved in DNA bending (*cf.* [26]).

We have started investigating, along similar lines, the effect of Hg(II) on $[d(G)_n \cdot d(C)_n]$ and $[d(G\text{-}C)_n \cdot d(G\text{-}C)_n]$ sequences.

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