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Mercury-Induced DNA Polymorphism: Probing the Conformation of Hg(II)-DNA via Staphylococcal Nuclease Digestion and Circular Dichroism Measurements†

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ABSTRACT: Exposing native calf thymus DNA to increasing concentrations of $Hg(ClO_4)_2$ not only produces dramatic changes in its circular dichroism (CD) but results also in the decrease, and ultimate cessation, of endonucleolytic DNA cleavage by staphylococcal nuclease. Let r = [moles of added Hg(II)]/[mole of DNA base]: the conservative CD spectrum of the DNA B-form becomes nonconservative in appearance at $0.01 < r < 0.12$ (resembling DNA in C-form geometry) and assumes the spectral characteristics of a left-handed DNA double helix at $0.12 < r \leq 1.0$. DNA cleavage proceeds at or near the rates exhibited by untreated DNA at $0 < r < 0.08$. At Hg(II) levels of $0.08 < r < 0.5$, the rate of DNA hydrolysis decreases monotonically with increasing Hg(II) concentrations, and at $r > 0.4$, DNA cleavage ceases. Both the CD changes and the changes in the rate of DNA digestion are totally reversible upon the removal of Hg(II), at least up to $r = 1.0$, demonstrating that Hg(II) keeps all base pairs in register. For comparison purposes, native calf thymus DNA was also treated with methylmercury [$CH_3Hg(II)$], an agent known to disrupt the secondary structure of DNA. The treatment yielded single-stranded methylmercurated DNA with preserved right-handed helix screwness. In addition, this DNA is digested by staphylococcal nuclease much more rapidly than double-stranded control DNA. Lastly, neither the CD nor the cleavage rate changes are reversible upon the removal of methylmercury. We interpret the Hg(II)-induced alterations in the CD of native calf thymus DNA, and the hydrolysis rate changes observed with staphylococcal nuclease, to indicate that Hg(II) either produces in DNA reversible B ↔ Z transitions, passing transiently through C-like conformations, or generates non-B-conformational structures of presumably left-handed geometry.

Hg(II)[1] is known to interact strongly and yet reversibly with the nitrogen binding sites of purines and pyrimidines (Katz, 1952; Thomas, 1954; Yamane & Davidson, 1961; Simpson, 1964; Nandi et al., 1965). It is believed that with native 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 =NHgN= configuration (sp hybridization) (Yamane & Davidson, 1961; Katz, 1963; Nandi et al., 1965).

The concept of a mercury-induced cross-linking of the Watson and Crick strands of DNA, without disturbing the

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[1] Hg(II) is to refer to divalent inorganic mercury, sufficiently ionized so as to bind to the bases of the DNA. Since the precise nature (as well as concentration) of all mercuric species potentially present in the buffer systems of this study is unknown, they are also collectively denoted Hg(II). Similar considerations apply to $CH_3Hg(II)$, the one difference being that methylmercury, albeit divalent, is monofunctional.

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alignment of opposing bases too severely, is in harmony with the finding that removal of the mercuric ions with suitable strong complexing reagents fully restores the biological activity of the DNA (Dove & Yamane, 1960). However, very little is known indeed about the precise nature of the conformation of the Hg(II)–DNA complexes.

In this paper, we report that staphylococcal nuclease (EC 3.1.31.1) offers the opportunity to probe the conformation of mercurated duplex DNA, since the enzyme, over a wide concentration range of mercury, is not inhibited, whether the element is present as an inorganic salt [Hg(II)] or organic compound [$CH_3Hg(II)$]. We will also show, in combination with the results of circular dichroism measurements,[2] that complexation of duplex DNA by Hg(II) results in reversible transitions between right-handed and left-handed polymer screwness and that the enzymatic activity of the endonuclease is strongly dependent on these chiroptical (conformational) changes. Identical experiments performed on DNA complexed by methylmercury supplement the study. Methylmercury is known to denature native DNA (Gruenwedel & Davidson, 1966, 1967; Gruenwedel, 1972, 1985; Fu & Gruenwedel, 1976), and, hence, methylmercurated DNA can serve as an alternate conformational reference.

Experimental Procedures

Calf thymus DNA (sodium salt), type I, was purchased from Sigma. Sodium perchlorate, HPLC grade, was obtained from Fisher Scientific; mercuric perchlorate was a product of Aldrich. Cacodylic acid was purchased from Sigma; sodium cacodylate and sodium cyanide were obtained from Matheson. Methylmercuric hydroxide and calcium perchlorate were products of Alfa. Staphylococcal nuclease, Foggi strain, (EC 3.1.31.1, lot 58C160) was purchased from Worthington. Its activity was 25 882 units/mg of protein. All other reagents were of analytical grade. Doubly deionized water was used throughout the investigation.

Calf thymus DNA was first dissolved in 0.1 standard saline citrate and then dialyzed exhaustively in the cold against 0.1 M $NaClO_4$ and 5 mM cacodylic acid buffer, pH 6.8, and stored over chloroform at 4 °C. Final DNA concentrations in the stock solution ranged from 0.5 (circular dichroism measurements) to 4.5 mg/mL (enzymatic digestion).

A mercuric perchlorate stock solution, about 0.1 M, was prepared by dissolving the appropriate amount of the salt in distilled water. The red precipitate of HgO, formed in the solution due to hydrolysis, was removed by filtration and the Hg(II) concentration in the filtrate determined via atomic absorption spectroscopy. Final Hg(II) concentration in the stock solution was 0.095 M. Other concentration levels were obtained by serial dilution into 0.1 M $NaClO_4$. The solutions were stored in the dark; no further precipitation of HgO was observed.

Methylmercuric hydroxide, obtained from Alfa as an approximately 1 M solution, was found to be 1.112 M in CH_3HgOH. The concentration was determined as described elsewhere (Gruenwedel & Davidson, 1966). Other concentration levels were obtained by serial dilution of the stock solution into 0.1 M $NaClO_4$.

Enzymatic digestion of DNA was executed as follows: DNA stock was diluted with buffer (vide infra) to 1.0 A^{260}. Two milliliters of this DNA (*a*) were then combined with 0.020 mL each of (*b*) the appropriate stock solutions of $Hg(ClO_4)_2$ or CH_3HgOH, (*c*) the enzyme (final enzyme concentration 6–7 units/mL), and (*d*) 0.2 M $Ca(ClO_4)_2$ (final concentration 2 mM). Mixing was performed in matched quartz cuvettes of 1-cm path length by gentle inversion. No differences in the rate of hydrolysis were noted when the sequence of addition [(a) + (b) + (c)] was changed to [(a) + (c) + (b)] or some other combination. All pipetting was done with calibrated pipets. Progression of DNA digestion was monitored at 260 nm in the Gilford 250 spectrophotometer, using the Gilford 6051 strip-chart recorder and the Gilford 2451A automatic cuvette positioner. Digestion occurred at 37 ± 0.2 °C (thermostated compartment). Temperatures were monitored with the help of the Fluke 52 K/J digital thermometer; a cuvette filled with buffer, located adjacent to the digestion cuvette, contained the thermocouple. The strip-chart recorder was left running during all manipulations (speed 1 cm/min), and the A^{260} base line was recorded for a couple of minutes each time after the addition of components b or c. Once component d had been added, monitoring of hydrolysis commenced (t = 0 min). A time span of maximally 5 s was needed to mix (d) with [(a) + (b) + (c)] and to return the mixture to the spectrophotometer chamber. Pen tracings were followed for 5–10 min, and rates were evaluated from the linear portion of the tracings. As a rule, linearity persisted for 1–5 min. DNA digestion was performed in two different buffer systems: buffer A, 0.1 M $NaClO_4$ and 5 mM cacodylic acid buffer, pH 6.8; buffer B, 0.1 M $NaClO_4$ and 5 mM boric acid buffer, pH 8.9. Digestions were also carried out in 0.1 M boric acid buffer, pH 8.9; however, since the hydrolysis rates determined in this buffer did not differ from those collected in buffer B, only the data pertaining to buffer B are presented. Rates measured in buffer A were smaller than those collected in buffer B, but they displayed the same dependence on Hg(II) or $CH_3Hg(II)$ concentration. The amount of DNA-bound mercury was varied either by adding more duplex DNA (a′) to the digestion mixture or by adding (e) NaCN (in varied molar excess over mercury). Components a, a′, b, c, d, and e were combined in different sequences to detect possible artifacts. None could be detected. The same techniques were employed when methylmercury was used; however, DNA digestion there took place only in buffers A and B. Rates of enzymatic digestion were evaluated as A^{260} units of released oligonucleotides min^{-1} $unit^{-1}$ of enzyme. It should be noted that our assay differs from the standard micrococcal nuclease assay [cf. *The Worthington Manual* (1988)] by employing the DNA substrate at $^1/_{14}$ of the level used ordinarily.

Mercury concentrations are expressed in terms of the normalized quantity r, where r = $[Hg(ClO_4)_2]/[DNA(P)]$ = $[CH_3HgOH]/[DNA(P)]$, i.e., the number of moles of mercury added per mole of DNA base, or in terms of pM, with pM = $-\log [Hg(ClO_4)_2]$ = $-\log [CH_3HgOH]$. The brackets, as usual, denote molar concentrations.

Circular dichroism (CD) measurements were performed by using the JASCO 500C spectropolarimeter in combination with the JASCO DP-501N data processor. Spectra were recorded at 25 °C from 360 to 200 nm, on occasion also down to 190 nm. Each run consisted of eight repeat scans, executed automatically, which increased the signal-to-noise ratio by a factor of $8^{0.5}$ = 2.83 (compared to a recording executed only once). Data collected after the photomultiplier voltage had exceeded 800 V were rejected. Recordings were executed with the following instrument settings: scan speed, 10 nm/min; time constant, 4 s; sensitivity, 2 mdeg/min. Ultrapure dry nitrogen,

[2] A preliminary account of the results of Hg(II)–DNA CD measurements was given by the senior author at the American Society for Cell Biology/American Society for Biochemistry and Molecular Biology Annual Meeting, San Francisco, Jan 29–Feb 2, 1989 (Gruenwedel, 1989).

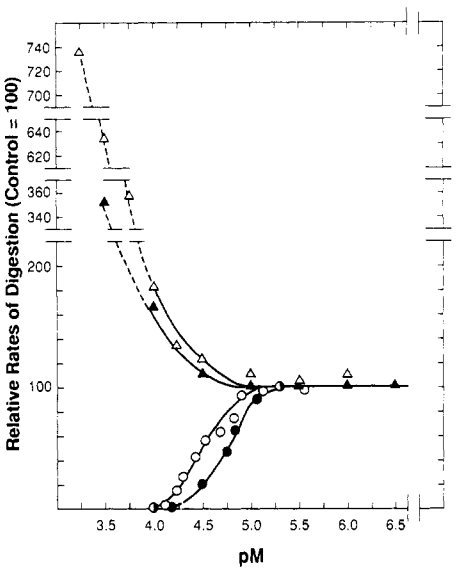

FIGURE 1: Effects of Hg(II) (circles) and $CH_3Hg(II)$ (triangles) on the rate of endonucleolytic DNA cleavage in the presence of staphylococcal nuclease. (Solid symbols) Rates determined in 0.1 M $NaClO_4$, 5 mM cacodylic acid buffer, pH 6.8 (buffer A). (Open symbols) Rates determined in 0.1 M $NaClO_4$, 5 mM boric acid buffer, pH 8.9 (buffer B) (the dashed tracings are to indicate that the scale of the rate axis had to be changed to accommodate all data points). Absolute rates of digestion (control DNA): buffer A, 0.002 A^{260} unit min^{-1} (enzyme unit)$^{-1}$; buffer B, 0.007 A^{260} unit min^{-1} (enzyme unit)$^{-1}$. pM = -log $[Hg(ClO_4)_2]$ added = -log $[CH_3HgOH]$ added. The brackets denote molar concentrations. Half-solid symbols are overlaps of open and solid symbols.

at flow rates ranging from 5 to 10 L/min, the higher flow rate applying to the low end of the wavelength range, was used to flush the instrument and to prevent buildup of ozone, which would damage the optics.

CD spectra were collected from the following mixtures: (f) DNA + solvent; (g) DNA + Hg(II) + solvent; (h) DNA + Hg(II) + NaCN + solvent; (i) Hg(II) + solvent; (j) NaCN + solvent; (k) Hg(II) + NaCN + solvent; (l) solvent. Similar combinations were recorded when $CH_3Hg(II)$ was used. Both buffers A and B served as solvents when Hg(II)–DNA interactions were monitored; in the case of $CH_3Hg(II)$, only buffer A was employed. Combinations of mixtures f–l were used to obtain solvent-corrected spectra; all corrections were undertaken electronically. Since all measurements were done with the same 1-cm path length cuvette, the cuvette always facing the same direction in the cell holder and being positioned always at the same height, the spectra were thus also corrected for cuvette effects. Final DNA concentrations were between 40 and 50 μg/mL. CD is expressed in terms of molar ellipticity $[\theta]$ (deg cm^2/dmol). Ammonium *d*-camphor-10-sulfonate was used to calibrate CD signals; neodymium glass was used in wavelength calibrations. A molar absorptivity of 6600 (L/mol(P)/cm) (at 260 nm) was used to calculate calf thymus DNA concentrations.

All manipulations involving Hg(II) or $CH_3Hg(II)$ were done under chemical hoods, and disposable gloves and automated pipets were used. Cuvettes were kept stoppered during CD and nuclease digestion measurements, and the measurements were performed in well-ventilated rooms. Further information concerning the safe laboratory use of mercury compounds can be found in Junghans (1983).

RESULTS

Staphylococcal Nuclease Digestion. As can be seen from Figure 1, treating native calf thymus DNA with increasing concentrations of $Hg(ClO_4)_2$ (circles) leads to a decrease in the rate of endonucleolytic DNA cleavage. The rate decrease occurs over a rather narrow concentration range of Hg(II): it starts at about 0.01 mM (pM 5) and is completed at about 0.1 mM (pM 4). Digestion was monitored at pH 8.9, the pH optimum of the enzyme (open circles), and at pH 6.8, the pH at which most of the circular dichroism measurements (vide infra) were performed (solid circles). While solvent influences on the rate changes can be noted in the figure, they are only of minor importance because the overall shape of the pH 6.8 and pH 8.9 transition curves remains the same. The differences may be due to pH, affecting both the activity of the enzyme and the availability of reactive Hg(II), and/or due to specific anion effects, e.g., complexation of Hg(II) by borate or cacodylate.

The decrease in the rate of DNA digestion is totally reversible: removing DNA-bound Hg(II) with NaCN (in 10-fold molar excess over mercury, for instance) fully restores the residual rate to that of the control. In fact, through judicious use of NaCN, one can bring about a partial abstraction of mercury from the DNA with a corresponding increase in endonucleolytic hydrolysis. The same can be achieved by simply adding untreated native calf thymus DNA to the mixture.

A totally different digestion pattern is observed when native calf thymus DNA is subjected to increasing concentrations of methylmercuric hydroxide (cf. Figure 1, triangles): first, higher concentrations of organic mercury are required to bring about rate changes. Indeed, it is for this reason that we plotted the logarithm of the mercury concentrations, and not the concentrations themselves, against the cleavage rates so as to accommodate the methylmercury concentrations in the same figure. Second, DNA cleavage rates increase rather than decrease upon the addition of CH_3HgOH (Figure 1, open triangles, buffer B; solid triangles, buffer A). Solvent effects can be noted also here: the rate of digestion is considerably higher at the pH optimum of the enzyme than at pH 6.8.

While we did not increase the methylmercury concentration beyond the pM value of 3.5 in buffer A, resulting in a 3.5-fold rate increase above control (Figure 1, solid triangles), we extended our measurements to pM 2.0 in buffer B: a rate maximum appears to exist at pM 3.0 (yielding an approximately 9-fold rate increase over control; not shown in the figure) but at pM 2.0, the rate of digestion dropped precipitously to a level at about 300% above the control value. We did not explore this finding any further in view of the health hazards posed by methylmercury at the higher concentrations.

With $CH_3Hg(II)$, the noted increases in cleavage rate are not reversible: complete abstraction of DNA-bound CH_3-Hg(II) with excess NaCN keeps the rate at or near the level prior to its removal.

To correlate better the results of the enzymatic digestion of DNA with the results of the circular dichroism measurements, we have also plotted the Hg(II)-induced rate changes against r, the number of moles of $Hg(ClO_4)_2$ added per mole of DNA base. This is shown in Figure 2. While the main graph displays the results obtained at pH 8.9 (buffer B), the inset pertains to pH 6.8 (buffer A). In buffer B, the addition of mercury has little effect on the rate of DNA digestion by staphylococcal nuclease at $0 < r < 0.08$. The rate decreases then in monotonic fashion with increasing r values and reaches zero near $r = 0.5$. A similar pattern is noted at pH 6.8, although the system's response is more sensitive. We did not deploy the rate increases found with $CH_3Hg(II)$ in terms of r units, for this would have yielded $r > 1$ at pM <4 and, hence,

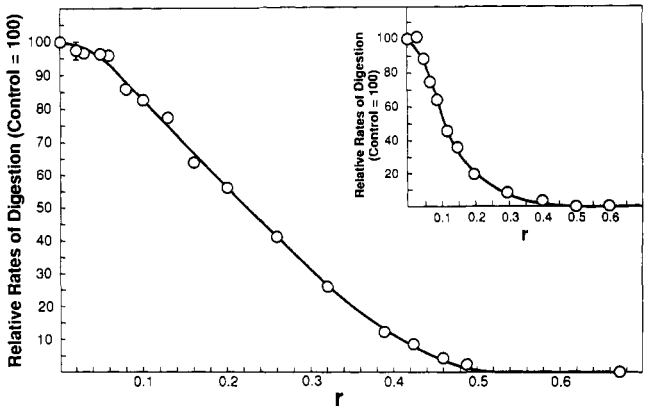

FIGURE 2: Effects of Hg(II) on the rate of endonucleolytic DNA cleavage in the presence of staphylococcal nuclease. (Main panel) Rates determined in buffer B; (inset) rates determined in buffer A (for buffer composition as well as absolute rates of digestion, the legend of Figure 1 should be consulted). r refers to the number of moles of Hg(II) added per mole of DNA base. The error bar shown for $r = 0.02$ (buffer B) indicates the maximum rate variations found in triplicate measurements. Similar rate variations were observed in buffer A. In either buffer, their width extends to about $r = 0.2$. At $r > 0.2$, variations become less noticeable; there, their spread falls within the boundaries of the symbols.

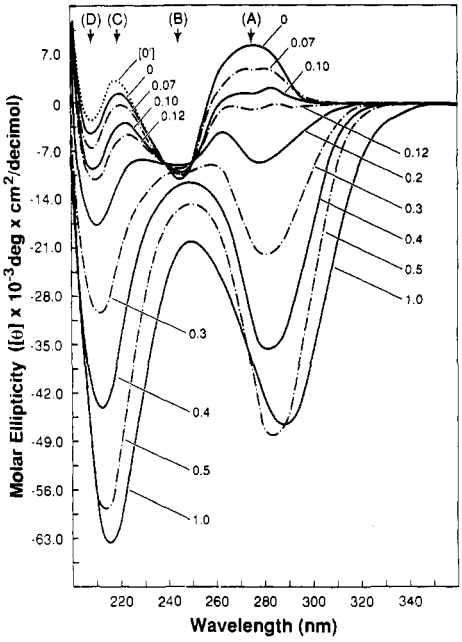

FIGURE 3: Circular dichroism (CD) spectra of native calf thymus DNA treated with Hg(II). The numbers with the curves are r values. Measurements were performed in buffer A (pH 6.8). For further details, see text.

been outside the range of the figure.

Circular Dichroism (CD) Measurements. In Figure 3 are assembled the circular dichroism spectra of native calf thymus DNA collected in buffer A and displayed as a function of r values. Untreated DNA ($r = 0$) shows the well-known features of a so-called conservative CD spectrum: (A), the major (+) band, situated between zero ellipticity points at 305 and 256 nm, has a molar ellipticity of $[\theta] = +8481$ at 273 nm; (B), the major (−) band, located between crossover points at 256 and 225 nm, has a $[\theta]$ value of −10645 at 245 nm; (C), the minor (+) band between zero ellipticity points at 225 and 214 nm, displays a $[\theta]$ value of +1872 at 219 nm; and (D), the minor (−) band, situated between crossover points at 214 and 204 nm, has a $[\theta]$ value of −3732 at 208 nm.

Changes in this CD spectrum are not noted at r values up to $r = 0.01$, i.e., the CD spectra collected at $r = 0.001$ or $r = 0.005$ are indistinguishable from the $r = 0$ spectrum. However, major alterations, particularly in bands A, C, and D, become noticeable at $r > 0.01$. Most striking is the collapse of the A band: its chirality has more or less vanished at r values near 0.1. By contrast, the ellipticity of the B band does not change very much with Hg(II) at $0.01 < r < 0.12$.

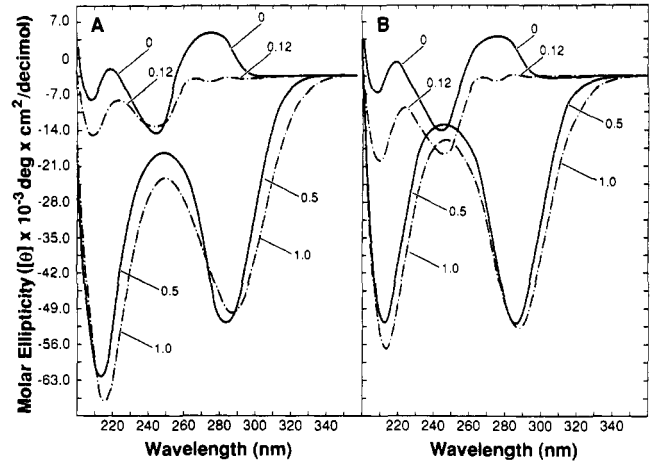

FIGURE 4: Circular dichroism (CD) spectra of native calf thymus DNA treated with Hg(II). (Panel A) Buffer A (pH 6.8); (panel B) buffer B (pH 8.9). The numbers with the curves are r values. For further details, see text.

Further addition of Hg(II) imparts negative chirality to the A band ($r > 0.12$), while the B band, after having shown some invariance with mercury between 230 and 250 nm ($0.12 < r < 0.4$), assumes "positive" CD characteristics. The C band merges with the inverted B band at $r > 0.3$, and the D band displays large negative ellipticity values. Indeed, at r values ranging from 0.5 to 1.0, the CD of mercurated DNA is more or less the mirror image of that of untreated DNA, albeit with an overall negative chirality. All CD changes are accompanied by red shifts; this has been noted by others when studying the effects of mercury on the ultraviolet absorption spectra of nucleic acids [cf. Thomas (1954), Yamane and Davidson (1961), Simpson (1964), and Gruenwedel and Davidson (1966)].

Removal of Hg(II) from the DNA with the help of NaCN reverts the CD back to that of untreated DNA (Figure 3): with the minor deviation noted at wavelengths below 225 nm (indicated by [0′] in the figure), the spectrum of untreated DNA is indistinguishable from that of "de-complexed" DNA.

Although almost all of the CD work was done in buffer A, the results apply equally well to buffer B. This is shown in Figure 4 for $r = 0$, 0.12, 0.5, and 1.0: apart from a slight shift of the CD signals in borate buffer to more positive ellipticity values than in cacodylate buffer at wavelengths below 260 nm, the overall features of the spectra are the same. It is possible that borate–deoxyribose interactions make themselves felt at wavelengths below 260 nm.

Figure 5 shows the CD spectra of calf thymus DNA collected in buffer A in the absence of $CH_3Hg(II)$ (pM ∞; $r = 0$) and in the presence of 0.032 mM $CH_3Hg(II)$ (pM 4.5; $r = 0.2$) as well as 0.32 mM $CH_3Hg(II)$ (pM 3.5; $r = 2.1$). It will be recalled that these are the organomercurial concentrations at which the increase in the rate of DNA digestion by staphylococcal nuclease either just becomes noticeable or proceeds rapidly (cf. Figure 1). It is seen immediately that these CD spectra differ totally from those obtained in the presence of Hg(II) (cf. Figures 3 and 4): although bands A and B, too, undergo the red shift upon the addition of methylmercury, and band A decreases somewhat in magnitude, there is no inversion of the sign of the first Cotton effect (viz.,

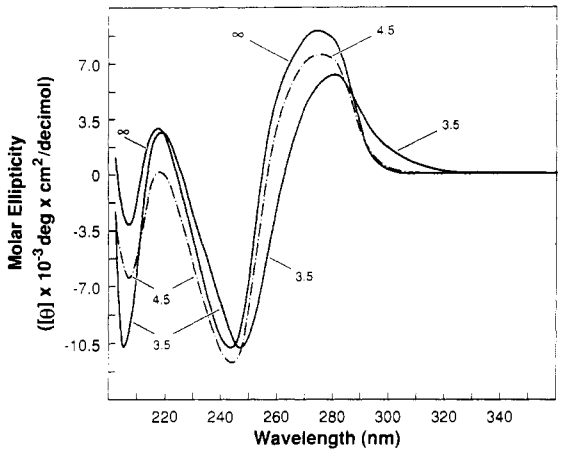

FIGURE 5: Circular dichroism (CD) spectra of (native) calf thymus DNA treated with $CH_3Hg(II)$. The numbers with the curves are pM values. Measurements were performed in buffer A (pH 6.8). For further details, see text.

band A) as noted with Hg(II). As shown elsewhere (Clegg & Gruenwedel, 1979; Gruenwedel, 1985), the methylmercury-generated CD spectrum at pM 3.5 is that of denatured DNA.

Discussion

Hg(II) interacts with ligands through coordination. Hence, it could attach itself to points of high electron density (e.g., σ electron pairs) in both DNA and staphylococcal nuclease. The hydrolysis rate data presented above are, however, internally consistent only if considered on the basis of mercury–DNA interactions, which, as we believe, result in alterations in DNA topology. If the kinetic data were due to mercury reacting with the enzyme, one would be faced with the curious result that while Hg(II) displays inhibitory properties, CH_3-Hg(II) activates the enzyme. We base our conclusion of substrate (rather than enzyme) effects on the following:

(*1*) The absence of sulfhydryl groups or disulfide bridges in staphylococcal nuclease (Cone et al., 1970) should render mercury–enzyme interactions less severe. That this is indeed the case is shown by the fact that the nuclease, in the absence of DNA and dissolved at a concentration of 0.06 mM in buffer A, does not experience alterations in its CD spectrum in the presence of Hg(II) at levels up to 0.3 mM (pM 3.52):[3] the four near-ultraviolet CD bands of staphylococcal nuclease retain their features with respect to shape, intensity, and wavelength position, demonstrating that mercury affects neither the enzyme's one tryptophan residue (weak positive CD band at 294 nm) nor the tyrosine residues (medium-strength negative band centered at 276 nm). Also, its α-helix content is not diminished judging from the invariance of the two strong negative bands at 220 and 208 nm with mercury [for CD band assignments, Omenn et al. (1969) should be consulted]. It will be recalled that Hg(II) at pM 3.5 totally abolished DNA cleavage by staphylococcal nuclease (Figure 1).

(*2*) By contrast, duplex DNA has a high affinity for Hg(II). As shown by Nandi et al. (1965), native calf thymus DNA, at a concentration of about 1.5×10^{-5} M in DNA-P and dissolved in 0.5 M $NaClO_4$ and 10 mM boric acid buffer, pH 8.95, binds mercury so strongly that the concentration of free Hg^{2+} amounts only to about 10^{-19} M at an r value of 0.4. Since the experimental conditions of our work (cf. buffer B) do not differ very much from those of Nandi et al. (1965), the concentration of free Hg^{2+} should also be negligible in the r range of our study.

(*3*) Staphylococcal nuclease is known to be sensitive to the topology of DNA, e.g., (left-handed) Z-form poly[d(G-C)·d-(G-C)] is much more resistant to digestion than the (right-handed) B-form (Möller et al., 1984). We find no or only little inhibition of digestion as long as the substrate displays right-handed helix screwness, but the rate of hydrolysis decreases substantially, and vanishes ultimately, once inversion of the first Cotton effect occurs (cf. Figures 2 and 3).

(*4*) Hg(II) is known to preserve the secondary structures of DNA. The complete restoration of enzymatic activity upon the removal of reactive Hg(II) from the DNA substrate by the strong complexing agent NaCN is consistent with this concept of reversible Hg(II)–substrate interactions. Further, instantaneous (i.e., within 5 s) and complete restoration of enzymatic activity is observed whether removal of mercury at $r = 1.0$ occurs at 37, 15, or 5 °C (results not displayed). This rules out enzyme denaturation by mercury [see also (*1*) above].

(*5*) $CH_3Hg(II)$ is known to convert double-stranded DNA to single-stranded DNA. Thus, the observed increase in the rate of hydrolysis is in harmony with this fact, for staphylococcal nuclease hydrolyzes single-stranded DNA preferentially [cf. von Hippel and Felsenfeld (1964)]. Since calf thymus DNA, in view of its compositional heterogeneity, does not renature readily upon the removal of any denaturing agent, it is not surprising that the rate of DNA cleavage, subsequent to the complexation of $CH_3Hg(II)$ by NaCN, remains at or near the high levels measured prior to the addition of NaCN.

(*6*) The rate of hydrolysis is governed by r, i.e., by the availability of DNA bases not complexed by mercury. The following shall illustrate this point: adjusting r to a value near 0.7 renders the DNA cleavage rate zero (cf. Figure 2). The rate remains at zero even after the amount of enzyme is increased 20-fold (e.g., to 120–140 units/mL). This is understandable because not enough uncomplexed DNA is left to be digested at noticeable rates. However, adding so much untreated calf thymus DNA to the mixture that r is lowered to, say, 0.3 reestablishes the rate of hydrolysis to the value valid at $r = 0.3$ or, in the presence of a 20-fold higher enzyme level (120–140 units/mL), to a rate that is about 20 times larger than the one measured in the presence of the enzyme level of 6–7 units/mL. Quite obviously, enough uncomplexed DNA was made available by the redistribution of Hg(II) so as to permit continued hydrolysis at measurable rates.

In conclusion, points 1–6 provide ample evidence that it is the conformational change of the DNA that accounts for the decrease in the rate of endonucleolytic DNA cleavage by staphylococcal nuclease and not an inactivation of the enzyme by the metal.

Turning now to the CD measurements, the CD signals displayed by duplex DNA at r values ranging from 0.01 to 0.1 (Figure 3) are in harmony with those obtained from DNA films in C-form geometry (Tunis-Schneider & Maestre, 1970). It appears reasonable that Hg(II), once inserted between base pairs, should affect DNA helix parameters [e.g., winding angle, shift, tilt (Johnson et al., 1981)] to such a degree that the alignment of the base pairs corresponds to that found in C-form DNA.

[3] We will report elsewhere in detail (Gruenwedel and Cruikshank, unpublished results) about the effect of Hg(II) on (a) UV absorption and CD of staphylococcal nuclease, (b) UV absorption and CD of thymidine 5′-monophosphate *p*-nitrophenyl ester (R-p-dT), (c) binding of R-p-dT to the enzyme, and (d) rate of hydrolysis, using R-p-dT as substrate.

From the fact that Hg(II) changes the sign of the first Cotton effect (band A in Figure 3) from (+) to (−) at $r > 0.1$, one is forced to conclude that inorganic mercury conveys left-handed screwness upon the electric transition dipole moments of neighboring nucleotides. Such left-handed screwness exists, for example, in Z-DNA (Rich et al., 1984; Jovin et al., 1987). That more than one nucleotide has to be present to bring about CD inversion may be deduced from the following: the four Cotton bands of thymidine 5′-monophosphate[3] (as *p*-nitrophenyl ester) are not inverted in the presence of $Hg(ClO_4)_2$ at $r = 1.13$ and 5.7, yet a slight red shift of the bands is noticeable. Inversion of CD bands is thus a consequence of the orientation of neighboring nucleotides in a polymer chain.

Whether the conformation of DNA at, say, $1.0 > r > 0.1$ is indeed that of Z-DNA, or that of a left-handed DNA in a non-Z conformation, remains to be seen. The results of the staphylococcal nuclease digestion experiments are certainly in agreement with mercurated DNA possessing a Z-like structure. It should also be noted that Hg(II), a d^{10} element, is completely achiral and does not contribute to the CD on its own. Hence, all spectral changes are due to changes in the CD of DNA.

Since in Z-DNA alternating residues adopt C3′-endo/syn and C2′-endo/anti geometries—in contrast to B-DNA where they all are anti—the anti position of, say, deoxyguanosine in B-DNA could easily be changed to the syn position by Hg(II) forcing the base to rotate around its glycosyl carbon–nitrogen linkage. This could be accomplished by Hg(II) binding to N(7) rather than to N(1)–H and, thus, interconnecting N(7) with some other binding site. Although, with free guanosine, Hg(II)'s affinity to N(7) is by about 2 orders of magnitude lower than to N(1)–H (at pH values near 7) (Simpson, 1964), it is possible that steric conditions favor the N(7) position in the double helix. As shown by Keller and Hartman (1987) via infrared spectroscopy on hydrated films of mercurated poly[d(G-C)·d(G-C)], N(7) [or N(3)] appears indeed to be the exclusive binding site(s) in the polynucleotide. Not surprisingly, they find the polynucleotide to assume the Z structure, at different relative humidities, with Hg(II) at levels of $0.2 < r < 0.6$. This is in good agreement with the CD inversions taking place at $r > 0.12$ (Figure 3).

The finding that the inversion of the CD A band reaches a limit near $r = 0.5$ (Figures 3 and 4) appears reasonable in view of the fact that mercury binds to DNA bases in the sequence T > G ≫ A, C [cf. Gruenwedel and Davidson (1966)] and that the mole ratio [T + G]/[A + T + G + C] in DNA equals 0.5.

Flow linear dichroism spectra of Hg(II)–DNA complexes (Matsuoka & Norden, 1983) were interpreted to originate with B-geometry DNA up to $r < 0.5$ but to signal-altered local conformations at $r > 0.7$. However, statements regarding the nature of these changed conformations were not made.

Studies employing the polymers poly[d(A-T)·d(A-T)], poly[d(G-C)·d(G-C)], and poly[d(T-G)·d(C-A)] as well as poly[d(A)·d(T)] and poly[d(G)·d(C)] are in progress to explore the chiroptical inversions in greater detail (Gruenwedel and Cruikshank, unpublished results). Preliminary results show that while the polynucleotides with purine–pyrimidine (Pu–Py) sequences undergo the Hg(II)-induced CD inversions, those with Pu–Pu or Py–Py sequences do not. It is generally believed that Z-DNA formation is favored in regions made up of Pu-Py sequences but not in sequences consisting solely of Pu–Pu or Py–Py. Without doubt, calf thymus DNA contains enough Pu–Py sequences to permit B → Z transitions.

On the other hand, since CD spectroscopy is prone to yield artifactual information concerning structure assignments (Tinoco et al., 1980), there is always the possibility that mercurated calf thymus DNA retains B-form geometry in spite of CD indicating a left-handed helix. For instance, left-handed screwness was observed with DNA films at low relative humidities and explained on the basis of optical interactions occurring in quasi-crystalline microdomains caused by closely packed DNA molecules (Tunis-Schneider & Maestre, 1970). If applicable to this work, mercury binding would have to result in in situ strand condensation. We have started investigating this possibility with the aid of dynamic light scattering. However, on the basis of the fact that Hg(II) does generate Z-form poly[d(G-C)·d(G-C)] (Keller & Hartman, 1987) and that staphylococcal nuclease, as shown in this study, ceases to digest mercurated DNA, we find it difficult to reconcile these, and the CD findings, with a DNA that is still of (topologically unchanged) B-form geometry.

Although a number of cations, e.g., Na^+, K^+, Li^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+}, appear to induce B ↔ Z transitions in DNA (Zacharias et al., 1982; Rich et al., 1984; Jovin et al., 1987), they need to be present at rather high and nonphysiological concentrations or require additionally the presence of dehydrating organic solvents. The Hg(II)-induced CD inversions observed here become already noticeable when there are about 10 mercuric ions per 100 bases.

Finally, the hydrolysis rate data presented by Cuatrecasas et al. (1967) for staphylococcal nuclease in the presence of Hg(II) agree fully with ours. The authors do not seem to have probed the noted rate inhibition for reversibility or, for that matter, the effect of Hg(II) on the circular dichroism of both enzyme and DNA substrate. Hence, their interpretation (assuming enzyme inactivation) differs from ours. Whether other heavy metals, similarly reported to be potent inhibitors of staphylococcal nuclease activity (Cuatrecasas et al., 1967) and known to generate Z-DNA, exert their influence via substrate modification remains to be seen.

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Sequence Specificity of the Deoxyribonuclease Activity of 1,10-Phenanthroline–Copper Ion†

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ABSTRACT: A statistical analysis of a data set composed of over 1600 scission events of DNA produced by the 2:1 1,10-phenanthroline–copper complex (OP–Cu) has demonstrated that the nucleotide 5′ to the site of phosphodiester bond scission is a primary influence in the kinetics of cleavage at any sequence position. The scission was less affected by the 3′ neighbor. For each of the sixteen possible dinucleotides, a kinetic parameter can be computed reflecting scission at the 3′ nucleotide. When used to predict the scission pattern of a DNA sequence not part of the present data set, correlation coefficients of about 0.6 between predicted and observed patterns were obtained.

The nuclease activity of 1,10-phenanthroline–copper (OP–Cu)[1] exhibits sequence specificity in its efficient scission of B-DNA although it oxidizes the deoxyriboses of all four bases (Sigman, 1986). The major reaction pathway of the nuclease activity is summarized in Figure 1 and has been supported by the isolation of the free bases, 3′ and 5′ phosphomonoester termini, and 5-methylenefuranone (Pope et al., 1982; Kuwabara et al., 1986; Goyne & Sigman, 1987). In addition, by use of 5′-labeled DNA, a reaction intermediate has been trapped which likely corresponds to the reaction intermediate C (Kuwabara et al., 1986; Veal & Rill, 1989). The chemistry of the cleavage reaction demands that the coordination complex attack the deoxyribose from the minor groove.

The sequence-dependent scission of B-DNA and the specificity of the reaction for secondary structure can be attributed to a kinetic scheme in which the reaction is funnelled through an essential noncovalent intermediate [$(OP)_2Cu^+$–DNA] (Sigman et al., 1979; Thederahn et al., 1989).

$$(OP)_2Cu^+ + DNA \overset{K_d}{\leftrightarrow} (OP)_2Cu^+\text{–}DNA \xrightarrow{H_2O_2} \text{nicked products} \quad (1)$$

Preferred sites of scission are adjacent to high-affinity binding sites for the coordination complex within the minor groove. The specific interactions between the DNA and the coordination complex which result in tight binding in the minor groove have not been identified, but certain features of the sequence preference have been investigated. For example, the scission rates for a given sequence are insensitive to the length of the DNA fragment (Yoon et al., 1988a); repetitive sequences within a fragment exhibit comparable digestion patterns (Law et al., 1987). Tri- and tetranucleotide sequences have been identified which are preferentially cut. Veal and Rill (1988, 1989) have found that the central A of TAT is strongly cleaved and that related sequences such as TGT, TAAT, TAG, TAG–pyrimidine, and CAGT are moderately preferred. The most strongly cut sequence within the Tyr T promoter is CATATC (Drew & Travers, 1984). Sequences composed of A·T stretches are preferentially cleaved (Suggs & Wagner, 1986).

In this paper, we summarize our attempts to develop a statistical approach to the analysis of the sequence-dependent reactivity of OP–Cu. The most important variable influencing scission rates is the nucleotide 5′ to the site of scission.

Experimental Procedures

Methods

Reaction Conditions for OP–Cu Scission. Uniquely labeled restriction fragments were digested as previously described (Kuwabara et al., 1986; Kuwabara & Sigman, 1987). All sequencing gels were calibrated with the G+A reaction (Maxam & Gilbert, 1980).

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[1] Abbreviations: OP–Cu, 2:1, 1,10-phenanthroline–copper complex; EDTA, ethylenediaminetetraacetic acid; MPE, methidiumpropyl-EDTA; DTT, dithiothreitol; bp, base pair; SD, standard deviation; Tris, tris(hydroxymethyl)aminomethane; PMA, phorbol 12-myristate 13-acetate.

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