Hg(II)-Induced Changes in DNA-Circular Dichroism: 
Reversible Transitions between Right-Handed and 
Left-Handed Screwwess

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Exposing native calf thymus DNA (in 0.1 M NaClO₄, 5 mM cacodylic acid buffer, pH 6.81, 25 °C) to increasing concentrations of Hg(ClO₄)₂ produces dramatic changes in its circular dichroism (CD). Let r = [mol of added Hg(II)]/[mol DNA base]; the conservative CD spectrum of the DNA B-form, consisting of the 273 nm major (+) CD band, the 245 nm major (−) CD band, the 219 nm minor (+) CD band, and the 208 nm minor (−) CD band, becomes non-conservative in appearance at 0.01 < r < 0.12 and assumes the spectral characteristics of a left-handed DNA double helix at 0.12 < r > 1.0. The presence of a number of isoelectricity points shows that well-defined equilibria exist between the various chirotical forms of mercurated DNA. The CD changes 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.

Introduction

Hg(II) is known to interact strongly and yet reversibly with the purine and pyrimidine residues of nucleic acids [1–6]. It is believed that with native DNA the metal is chelated between the Watson-Crick base pairs, forming strong bonds to the sigma electron pairs of nitrogen atoms in a linear =N—Hg—N= configuration (sp-hybridization). Since removal of mercury from the DNA fully restores its biological activity [7], it has been held that Hg(II) not only keeps all base pairs in register but maintains the B-form geometry of DNA as well.

As shown in this contribution, Hg(II) produces tremendous changes in the circular dichroism of native DNA. The changes from positive to negative chirality — demonstrating major perturbations in the electric transition dipole moments of the constituent bases — may be interpreted as a sequential change of DNA from a right-handed to a left-handed double helix. Whether this amounts to a B-DNA to Z-DNA transition, or to a non-Z-conformational change, remains to be seen; however, the assumption that complexing of DNA by Hg(II) does not affect its B-form geometry clearly is no longer tenable.

Materials and Methods

Calf thymus DNA (sodium salt), Type I, was purchased from Sigma. All other chemicals were of analytical grade. Doubly-deionized water was used throughout the investigation.

Calf thymus DNA, dissolved at a final concentration of 40—50 µg/ml in 0.1 M NaClO₄, 5 mM cacodylic acid buffer, pH 6.81, was combined with appropriate quantities of mercuric perchlorate, also dissolved in 0.1 M NaClO₄, 5 mM cacodylic acid buffer, pH 6.81**. Hg(II) concentrations were determined via atomic absorption spectroscopy. Final Hg(II) concentrations are expressed in r-values, with r = [Hg(II)]_added/[DNA(P)], or as pHg = -log[Hg(II)]_added.

Circular dichroism 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—200 nm. Each run consisted of eight repeat scans, executed automatically, which increased the

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** Since the nature (as well as concentration) of all mercuric species potentially present in 0.1 M NaClO₄ and at pH 6.81 is unknown, they are collectively denoted by Hg(II).

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signal-to-noise ratio by a factor of $(8)^{0.5} = 2.83$ (compared to a recording executed only once). All spectra were corrected for solvent and cuvette effects. Primary results are expressed in terms of molar ellipticity $[\theta]$ (deg cm$^2$/decimol). D-Camphor-10-sulfonate (ammonium salt) was used to calibrate CD signals.

Staphylococcal nuclease (EC 3.3.1.1) digestion experiments were executed at 37 °C in the Gilford Model 250 spectrophotometer. Digestion was monitored at 260 nm using published procedures [8].

Results and Discussion

Native calf thymus DNA, in absence of Hg(II), shows from 360–200 nm four CD bands (Fig. 1, $r = 0$): [A], the major (+) band, situated between zero-ellipticity points at 305 and 256 nm, with a molar ellipticity of $[\theta] = +8,487$ at 273 nm; [B], the major (−) band, located between cross-over points at 256 and 225 nm, with a [θ]-value of −10,661 at 245 nm; [C], the minor (+) band between zero-ellipticity points at 225 and 214 nm, with a molar ellipticity value of $[\theta] = +1,862$ at 219 nm; and [D], the minor (−) band, situated between zero-ellipticity points at 214 and 204 nm, with a molar ellipticity of $[\theta] = −3,723$ at 208 nm. This conservative CD spectrum is not affected by Hg(II) at levels up to $r = 0.01$, i.e., with less than one mercuric ion present per one hundred nucleotides.

Changes in the CD become noticeable at $r > 0.01$. Shown in Fig. 1 are the spectra collected at $r = 0.05$, 0.07, 0.09, and 0.12. Large changes can be seen in bands [A], [C], and [D]. Particularly significant is the collapse of the [A] band; by contrast, the major (−) band [B] is mostly unaffected by mercury.

Raising the Hg(II) levels above $r = 0.12$ produces the changes shown in Fig. 2: band [A], more or less abolished in the presence of about ten mercuric ions per one hundred nucleotides, now turns into a (−) band whereas band [B] assumes “positive” characteristics. Band [C] merges with band [D] at $r > 0.3$.

At $r$-values ranging from 0.5 to 1.0, the CD of mercurated DNA is more or less the mirror-image of untreated DNA, albeit with an overall negative chirality.

Three sets of iso-ellipticity points are noted from $r = 0$ to $r = 0.07$; there is one well-defined point at 291 nm and two, somewhat less discernible “regions” at 251 and 234 nm (Fig. 1). While the 292 nm point

![Fig. 1. CD of calf thymus DNA in 0.1 M NaClO$_4$, 5 mm cacodylic acid buffer, pH 6.81. The numbers with the curves refer to the number of mercuric ions per base ($r$); the letters denote the bands of the conservative CD spectrum at $r = 0$. For details, see text.](image1)

![Fig. 2. See legend to Fig. 1.](image2)
vanishes at $r > 0.07$, the 251 and 234 nm “regions” move to 249 and 237 nm, respectively. They are constant in the concentration range from $r = 0.09$ to $r = 0.2$ (Fig. 1 and 2). At higher Hg(II) concentrations, i.e., $r > 0.2$, they disappear also but a new iso-ellipticity point materializes at 270 nm. It holds for the spectra pertaining to $r = 0.5$, 0.74, and 1.0 (Fig. 2, the $r = 0.74$-curve has been omitted from the figure for reasons of clarity).

It appears that calf thymus DNA, upon complexation with Hg(II), passes through the following conformational stages: $B \rightarrow B'$ ($0 < r < 0.09$) $\rightarrow B''$ ($0.07 < r < 0.5$) $\rightarrow Z$ (or left-handed non-Z) ($r > 0.4$ up to $r = 1.0$). Both $B'$ and $B''$ are to represent modified DNA structures, still belonging to the B-family, with right-handed helix sense. They may, or may not, be equivalent to C-form DNA [9]. The fact that the transitions between the various iso-ellipticity points are rather sudden shows that formation of one particular conformational intermediate (e.g., $B'$) is essentially complete before formation of a new one begins (e.g., $B''$).

Removal of Hg(II) from the DNA, for instance, with the help of cyanide ions, reverts the CD back to that of untreated DNA (Fig. 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.

The CD signals displayed by duplex DNA at $r$-values near 0.09 (Fig. 1) are indeed in harmony with those obtained from DNA films in C-form geometry [9]. It seems reasonable that mercury, once inserted between base pairs, should affect DNA helix parameters (e.g., winding angle, shift, tilt) to such a degree that the arrangement of the base pairs corresponds to that found in C-DNA [10].

Less readily explained are the CD signals produced by mercurated DNA at $r > 0.1$. From the fact that Hg(II) changes the sign of the first Cotton effect from (+) to (−) (Fig. 2, band [A], $r > 0.1$) one is forced to conclude that mercury conveys a left-handed screwness upon the electric transition dipole moments of neighboring nucleotides. Such left-handed screwness exists, for example, in Z-DNA [11—13]. Whether the conformation of DNA between, say, $1.0 > r > 0.4$ is indeed that of Z-DNA, or that of a left-handed DNA in a non-Z-conformation, remains to be seen. In fact, left-handed screwness was also observed with DNA films (assumed to be still of B-form geometry) at low relative humidities and explained on the basis of optical interactions occurring in quasi-crystalline microdomains brought about by closely packed DNA molecules [9]. If correct, mercury binding by DNA would have to result in in situ strand condensation. We have embarked on a dynamic light scattering study to see whether mercuriation produces compact condensed forms of DNA. In any case, precipitation of the Hg(II)-DNA complexes, visible to the naked eye, does not occur.

It needs to be pointed out that all observed CD effects are Hg(II)-specific: thus, methylmercury (CH$_3$Hg(II)) alters the chiroptical properties of calf thymus DNA in a totally different pattern; most importantly, methylmercury preserves the right-handedness of the DNA [14].

That Hg(II) creates topologically novel DNA structures may also be deduced from the observation that staphylococcal nuclease (EC 3.31.1.) digests native calf thymus DNA with maximal rates between

![Fig. 3. CD of calf thymus DNA: in absence of mercury ($r = 0$), in presence of mercury ($r = 1.0$), and subsequent to the addition of a ten-fold molar excess of NaCN ([NaCN]/[Hg] = 10) ($r = [0']$). For details, see text.](image-url)
Table I. Staphylococcal nuclease digestion parameters of calf thymus DNA.

| pH     | $r^b$ | $R^c$ | Comments                  
|--------|-------|-------|---------------------------
| $\infty$ | 0     | 1.00  |                           
| 5.34   | 0.03  | 1.02  | Hg(II)$^d$                
| 5.12   | 0.05  | 0.89  | Hg(II)                    
| 4.86   | 0.09  | 0.64  | Hg(II)                    
| 4.64   | 0.15  | 0.33  | Hg(II)                    
| 4.34   | 0.30  | 0.08  | Hg(II)                    
| 4.21   | 0.40  | 0.01  | Hg(II)                    
| 4.12   | 0.50  | 0     | Hg(II)                    
| 5.00   | 0.07  | 1.02  | Me-Hg(II)$^e$             
| 4.50   | 0.21  | 1.10  | Me-Hg(II)                 
| 4.00   | 0.65  | 1.68  | Me-Hg(II)                 
| 3.50   | 2.07  | 3.55  | Me-Hg(II)                 

$^a$ Measurements performed in 0.1 M NaClO$_4$, 5 mM cacodylic acid buffer, pH 6.81. DNA concentration 50 ng/ml; enzyme concentration 7 units/ml; calcium concentration 2 mM.

$^b$ Definitions of pHg and $r$ are given in Materials and Methods section.

$^c$ Relative rate $R$ (with respect to control). Absolute rate (of control): 0.002 absorbance units at 260 nm/min/enzyme unit.

$^d$ Measurements performed in presence of Hg(ClO$_4$)$_2$.

$^e$ Measurements performed in presence of CH$_3$HgOH.

Since in Z-DNA alternating residues adopt C3'-endo/syn and C2'-endo/anti conformations – in contrast to B-DNA where they all are in 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 done by Hg(II) binding to N-(7) rather than to N-(1)-H. Although, with free guanosine, Hg(II)’s affinity to N-(7) is by about two orders of magnitude lower than to N-(1)-H (at pH-values near 7) [5], it is possible that steric conditions favor the N-(7) position in the double helix. As noted by Keller and Hartman [16], based on the results of infrared spectroscopy on hydrated films of poly[d(G-C) • d(G-C)], N-(7) of deoxyguanosine appears to be the exclusive binding site for Hg(II) in the polynucleotide, and in the concentration range 0.2 < $r$ < 0.6, they find the synthetic DNA to assume the Z-structure at different relative humidities. Hence, Hg(II) can indeed force deoxyguanosine to assume the C3'-endo/syn position in a double helix. This should occur if complexation results in less favorable stacking and base-phosphate interactions in the B structure.

Finally, although the chiroptical changes noted with calf thymus DNA subsequent to the addition of Hg(ClO$_4$)$_2$ strongly suggest that the polymer assumes ultimately a left-handed helix conformation, additional studies, employing, for instance, infrared/Raman or nuclear magnetic resonance spectroscopy, are needed to verify that this indeed the case. This is due to the fact that CD-signals do not always produce structurally correct answers [17].

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