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

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Exposing native calf thymus DNA (in 0.1 M $NaClO_4$, 5 mM cacodylic acid buffer, pH 6.81, 25 °C) to increasing concentrations of $Hg(ClO_4)_2$ 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 isoellipticity points shows that well-defined equilibria exist between the various chiroptical 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.

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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 $NaClO_4$, 5 mM cacodylic acid buffer, pH 6.81, was combined with appropriate quantities of mercuric perchlorate, also dissolved in 0.1 M $NaClO_4$, 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

* Since the nature (as well as concentration) of all mercuric species potentially present in 0.1 M $NaClO_4$ and at pH 6.81 is unknown, they are collectively denoted by Hg(II).

** $Hg(ClO_4)_2$, at higher concentrations in the buffer, yielded a precipitate of HgO due to hydrolysis. This was filtered off, and the concentration of mercury was determined in the clear filtrate prior to its combining with the DNA stock solution. No further precipitation was noted in the solutions.

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 [θ] (deg·cm²/decimol). D-Camphor-10-sulfonate (ammonium salt) was used to calibrate CD signals.

Staphylococcal nuclease (EC 3.31.1) 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 [θ] = +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 [θ] = +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 [θ] = −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

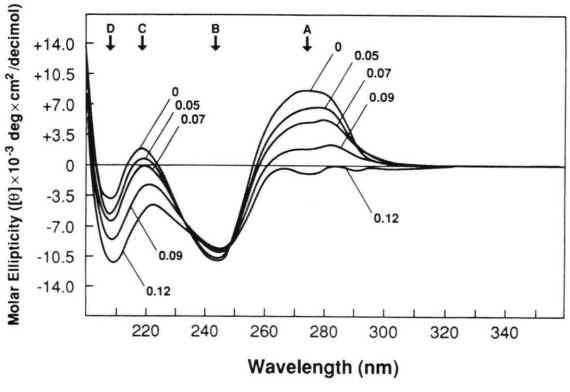

Fig. 1. CD of calf thymus DNA in 0.1 M $NaClO_4$, 5 mM cacodylic acid buffer, pH 6.81. The numbers with the curves refer to the number of mercuric ions per base (r); the letters denote the bands of the conservative CD spectrum at $r=0$. For details, see text.

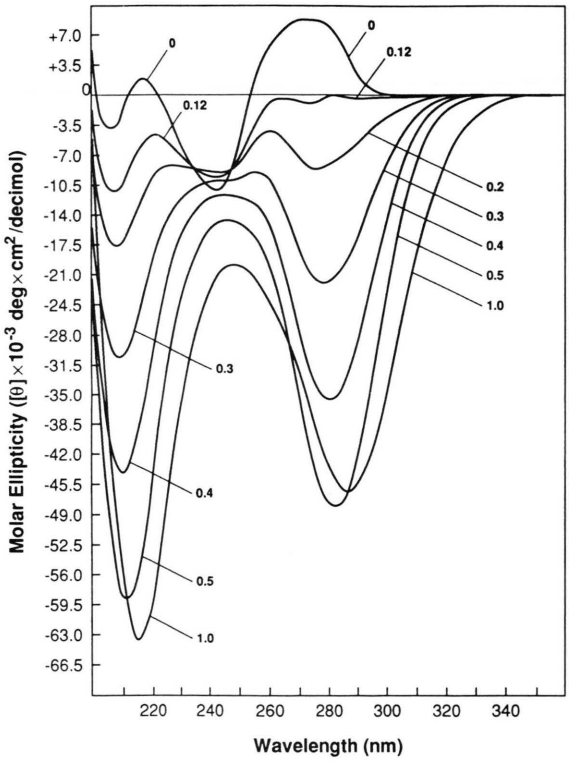

Fig. 2. See legend to Fig. 1.

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 though the following conformational stages: B → B′ ($0 < r < 0.09$) → B″ ($0.07 < r < 0.5$) → *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″).

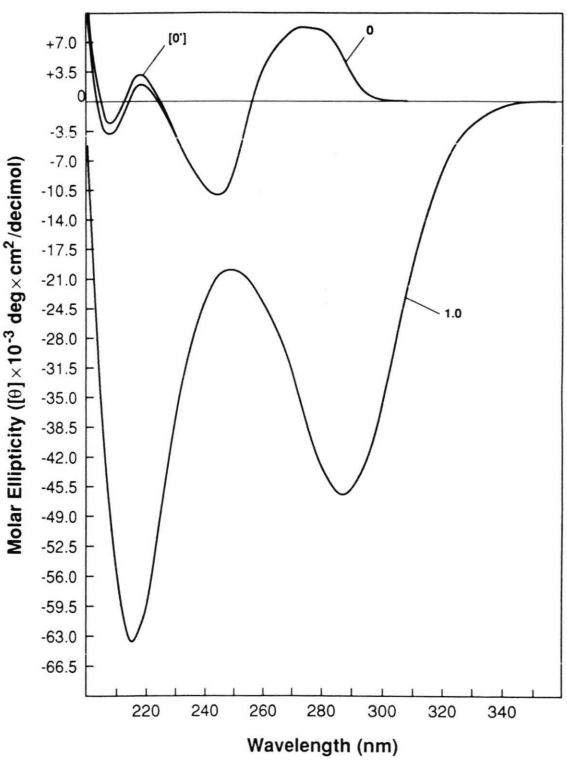

Fig. 3. CD of calf thymus DNA: in absence of mercury ($r = 0$), in presence of mercury ($r = 1.0$), and subsequent to the addition of a ten-fold molar excess of NaCN ([NaCN]/[Hg] = 10) ($r = [0']$). For details, see text.

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 mercuration 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 ($CH_3Hg(II)$) 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

Table I. Staphylococcal nuclease digestion parameters of calf thymus DNA[a].

pHg[b]	r[b]	R[c]	Comments
∞	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 μg/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_3HgOH.

$0 < r < 0.1$ but ceases to digest the substrate at $r > 0.4$. This is shown in Table I. Abstracting mercury from the DNA, either by adding mercury-free DNA in excess or an excess of NaCN, fully restores nuclease activity. Quite obviously, the decrease in the activity of enzymatic DNA digestion is due to conformational changes in the substrate (DNA) and not due to the inhibition of the enzyme by Hg(II). It is known that *Z*-DNA is much more resistant to digestion by staphylococcal nuclease than B-DNA [12]. Incidentally, methylmercury, at comparable concentrations, leads to an increase in the rate of DNA digestion by the enzyme (*cf.*, Table I). Methylmercury is known to produce single-stranded DNA [15], and single-stranded DNA is known to be digested preferentially by micrococcal nuclease.

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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