Interactions of Hg(II) ions with DNA as revealed by CD measurements

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ABSTRACT

The circular dichroism spectra of Hg(II) complexes with native calf thymus DNA, chemically methylated *Streptomyces chrysomallus* DNA and with Ag(I)-DNA complexes were measured in the region of 220 - 340 nm. As a main result a conversion of the conservative CD spectrum of DNA to a distinct nonconservative type of CD spectrum for the complexes occurs with increasing Hg(II) concentration. The CD spectra of the Hg(II) complexes as well as some additional arguments strongly support the idea, that DNA in the complex undergoes a structural transition to a more condensed state with ψ -like character.

INTRODUCTION

Among metal ions mercury(II) and silver(I) show the strongest interactions with nucleic acids /1,2/. Hg(II) like Ag(I) ions bind to the bases of nucleosides, polynucleotides, DNA and RNA and it is generally assumed, that probably no binding to phosphate groups exists /3/.

Pronounced structural changes in DNA complexes with Hg(II) have been demonstrated by various methods /4,5,6,7,8/. Optical rotatory dispersion (ORD) measurements of the DNA-Hg(II) complex have been performed in the visible spectral region by Pour-El /9/ and in the UV region by Cheng /10/ and Luck and Zimmer /11/. These authors found strong conformational changes indicated by negative Cotton effects in the UV range. Aktipis and Martz /12/ observed isosbestic points in the CD spectra of the DNA-Hg(II) complexes at low ratios of Hg(II) to DNA-phosphate. The existence of these isosbestic points depends on the GC content of DNA and confirmed the preferential binding of Hg(II) to AT pairs /6/.

At first Simpson and Sober /13/ published a strong non-conservative CD spectrum of the complex with negative maxima at $\lambda = 217$ nm and $\lambda = 285$ nm for excess of Hg(II) to DNA-phosphate. Nonconservative CD spectra with large negative maxima were observed likewise for DNA-Ag(I) complexes at about 270 nm, as reported by Ivanov et al. /14/ and recently for polynucleotide-Ag(I) complexes by Arya and Yang /15/. But aspects of ligand induced conformational changes by Hg(II) ions were not studied in detail by means of such a sensitive method as circular dichroism (CD) spectroscopy.

In the present work the transition from the conservative CD spectrum of DNA to a nonconservative CD spectrum on Hg(II) binding is described. The strong nonconservative CD spectrum observed at ratios around 1 Hg(II)/2 DNA-P is interpreted in terms of a condensed ψ -like state of DNA generated by complexing with Hg(II).

EXPERIMENTAL

DNA samples employed in these studies are those described by Sarfert and Venner /16,17/. Molar extinction coefficients of $\epsilon(P) = 6600$ (M.cm) $^{-1}$ for calf thymus DNA and $\epsilon(P) = 6200$ (M.cm) $^{-1}$ for Streptomyces chrysomallus DNA at 260 nm were used. Sedimentation coefficients of the samples are 23 S and 20 S respectively in 1 SSC. DNA from Str. chrysomallus with methylation degrees of 9.1, 24.1 and 41.8 % of guanine residues was prepared as previously described /18/. DNA stock solutions with a concentration of about 0.2 mg/ml were exhaustively dialysed against buffered solutions of sodium acetate (6 mM to 60 mM, pH 6.0) in the cold (4°C). The dialysate was diluted to concentrations appropriate for optical measurements (30-50 μ g/ml). All chemicals were reagent grade.

UV absorption measurements were performed with a Zeiss-spectrophotometer model Specord (VEB Carl Zeiss, Jena). CD spectra were recorded in a Cary 60 spectropolarimeter equipped with a 6001 CD attachment using 1 cm cells. The CD data are presented as circular dichroism per mole of nucleotide, $(\epsilon_L - \epsilon_R) = \theta/32.98.l.c$ in units of (M.cm) $^{-1}$, where ϵ_L and ϵ_R are the molar extinction coefficients for left and right

circularly polarized light. θ is the measured ellipticity in degrees, l is the pathlength in cm and c is the concentration of nucleotide subunits in mole/l = M. All measurements were performed at room temperature (22-25°C). For titration experiments $\text{Hg}(\text{CH}_3\text{COO})_2$ (10^{-3} M) was added in microliter quantities to a total volume of 2 or 4 ml of DNA. Bidistilled, deionized water as solvent and fresh Hg(II) solutions were used throughout the experiments. All solutions were stirred by nitrogen bubbling. In the experiments a relative concentration range from $r_t = 0$ to about $r_t = 2$ was covered, where r_t denotes the total amount of $\text{Hg}(\text{CH}_3\text{COO})_2$ added per nucleotide phosphate.

RESULTS AND DISCUSSION

Due to complexing of DNA with Hg(II) ions, the conservative CD spectrum of DNA is converted into a nonconservative spectrum, as shown in Fig.1 (A,B). The positive maxima of the DNA CD spectrum at 275 nm and at 220 nm decrease with increasing ratio r_t . Even at 1 Hg(II) per 100 DNA-P an initial reduction of the positive CD bands is measurable at low salt concentration (Fig.1A).

At $r_t \sim 0.17$ the positive CD band is no longer present and a negative CD maximum is formed which is associated with a bathochromic shift towards 285 nm. At further additions of Hg(II) up to $r_t = 0.8$ (Fig.1B) the metal binding to DNA generates an anomalous CD spectrum whose shape and largest negative amplitudes are strikingly similar to that of a ψ -type CD spectrum reported for DNA in systems of organic polymer solutions /19,20/. Extremely high r_t values cause a further bathochromic displacement and a diminution of the negative band, an effect which is not further studied in the present work. It should be noted that the negative CD maximum around 245 nm practically remains unchanged up to $r_t \sim 0.3$ whereas for higher r_t values a broad negative maximum centered at 250 nm is attained. For $r_t < 0.3$ an isosbestic point seems to exist around 250 nm which closely agrees with the findings of Aktipis and Martz /12/.

Figure 1: CD spectra of calf thymus DNA-Hg(II) complexes in 6 mM CH_3COONa , pH 6.0. Values of r_t are indicated for the complexes. A) $r_t = 0 - 0.26$; B) $r_t = 0; 0.26 - 1.20$.

Fig.2 shows the variation of the CD changes with increasing Hg(II) ion concentration recorded at four selected characteristic wavelengths. In this figure the difference CD, written as $\Delta(\epsilon_L - \epsilon_R) = (\epsilon_L - \epsilon_R)_{\text{DNA}} - (\epsilon_L - \epsilon_R)_{\text{Complex}}$, is plotted as a function of the ratio r_t at two salt concentrations. There are no significant differences between 6 mM and 60 mM sodium acetate. The curves for 285, 275 and 265 nm are s-shaped, i.e. their slope does not decrease monotonically with increasing r_t . Such a dependence is characteristic of cooperative binding if it can be assumed, that the measured signal

Figure 2: Changes of the CD amplitudes of calf thymus DNA by Hg(II) complexing for two salt concentrations at fixed wavelengths. CD spectra: 245 nm - minimum of DNA; 265 nm -; 275 nm - maximum of DNA; 285 nm - minimum of the complex.

is proportional to the concentration of the complex. A co-operative binding of Hg(II) to DNA has been described for a complex with 1 Hg(II) bound per base pair ($r \leq 0.5$) by Nandi et al. /7/ too.

The difference CD $\Delta(\epsilon_L - \epsilon_R)$ at 285 nm decreases for $r_t > 0.7 - 0.8$ (Fig.2) and probably reflects the formation of a further type of complex ($r > 0.5$) /5/, even if intermolecular aggregation cannot be excluded in this range of r_t .

In the range $r \leq 0.5$ Hg(II) binding to DNA is consistent with an AT-base specificity /6/. Thus the results in Fig.2 may be interpreted in terms of two species of complex formation represented by an AT specific and a second GC specific type of interaction. The latter species of the complex is clearly reflected by the CD changes at 245 nm. In this case a slight decrease in $\Delta(\epsilon_L - \epsilon_R)$ occurs up to $r_t = 0.25 - 0.30$ (Fig.2)

while for higher r_t 's an increase occurs. Moreover in the same r_t interval the isosbestic point around 250 nm vanishes. This finding is in accordance with Katz /21/, who discussed two possible types of complexes in the range $r \leq 0.5$ and suggested that the first species is exclusively dependent on the thymine content of DNA.

Therefore it was of interest to test Hg(II) binding to modified DNA with respect to possible alterations in GC selective binding effects. We have used a DNA of high GC content (Str.chrysomallus, 72 mole-% GC), which was chemically methylated to different degrees (0, 9.1, 24.1 and 41.8 % of guanine residues). As shown in Fig.3A the negative CD band at 285 nm of the Hg(II) complex ($r_t = 0.63$) decreases with increasing methylation of the guanine residues at the N-7 position. This diminution of the CD amplitude of the complex must not be only a consequence of reduced Hg(II) binding ability to methylated GC base pairs but also may arise from structural alterations of the DNA caused by methylated guanine residues (the highest methylated DNA may involve partially denatured regions) /18/.

Another possibility to check GC selective binding effects of Hg(II) is suggested by competitive interaction studies between Hg(II) and a metal ligand such as Ag(I), which binds specifically to the GC base pairs of DNA up to a ratio of 1 Ag(I) per 4 DNA-P ($\bar{r} = 0.25$) /22, 23/. Ag(I) at a ratio $\bar{r}_t = 0.2$ of added Ag(I)/DNA-P produces strong changes in the CD spectrum of DNA as shown in Fig.3B. An addition of Hg(II) with $r_t = 0.3$ to this Ag(I)-DNA complex generates a strong nonconservative CD spectrum with a negative band at 280 nm.

This CD spectrum cannot be generated by superposition of the two displayed CD spectra of the Ag(I)-DNA complex ($\bar{r}_t = 0.2$) and of the Hg(II)-DNA complex ($r_t = 0.3$), but this spectrum resembles the CD spectra of the Hg(II) complexes which are characteristic for r_t values at about 0.5 (Fig.3B). The position of the band maximum at 280 nm indicates that both Ag(I) and Hg(II) are bound to DNA, since the Ag(I)-DNA complex at $\bar{r}_t = 0.5$ has a negative maximum at 270 nm /14/ and the Hg(II)-DNA complex has one at 285 nm and both are of comparable amplitudes. At further addition of Hg(II) ($\bar{r}_t = 0.2$;

Figure 3: CD spectra of modified *Str.chrysomallus* DNA-Hg(II) complexes in 20 mM CH_3COONa , pH 6.0.

A) Chemically methylated DNA. Methylation degrees and the values of r_t are given.

B) Ag(I)-DNA complex with $\bar{r}_t = 0.2$. Values of r_t and the EDTA concentration are given.

$r_t = 0.6$) the amplitude of the negative maximum increases and the band shifts to 285 nm. For comparison the CD spectrum of a "pure" Hg(II)-DNA complex ($r_t = 0.7$) is included in Fig.3B.

The results are in accordance with a GC specificity of Ag(I) and an AT specificity of Hg(II). The CD spectral /14/ and hydrodynamic /6,7,8,22,23/ alterations of both DNA-cation com-

plexes are comparable with respect to type and order of magnitude. Apparently, Ag(I) and Hg(II) are able to induce similar structural changes of DNA. Recently, the formation of cross-links between the both strands of DNA by Ag(I) ions has been demonstrated by X-ray diffraction studies /24/. As long as only one cation per base pair is available, the structural changes of DNA due to Ag(I) plus Hg(II) binding are not independent of each other, but they obviously depend on the total amount of binding sites occupied by both competitive cations. Increasing the Hg(II) ion concentration so that the ratio of both cations per DNA-P reaches values above 0.5 leads to substitution of Ag(I) by Hg(II) in the DNA complex. This process is completely reversible, since the CD spectrum of the Ag(I)-DNA complex can be restored on addition of EDTA as a strong chelating agent for the divalent Hg(II) ions (Fig.3B). These findings also support the Hg(II) binding models with cross-links between the two opposite strands of DNA /7,21/ according to which a GC binding occurs after saturation of the AT sites.

The distinct CD spectral alterations indicate considerable distortions of the DNA helix by complex formation with Hg(II) ions. The question arises whether in the first binding steps defined conformational changes do occur which can be compared with well-characterized families of DNA conformation. Indeed, the decrease of the positive CD band and the approximate constancy of the negative band of DNA by Hg(II) binding in the range $r_t \leq 0.25$ (Fig.1A) closely resemble the behaviour demonstrated for the transition from B to a C-like DNA structure by Ivanov et al. /25/ at extremely high alkali salt concentrations. Similar observations and conclusions have been made too for binding of copper(II) /26/ and other transition metal ions to DNA /27,28/. We are tempted to interpret these CD changes likewise by a B to C-like conformational change. It is quite possible that the Hg(II) interactions might cause changes in the helix winding angle like those occurring in the conformational transition from B to C-like structures. Both crosslinks between bases of the two opposite strands of the DNA double helix according to a chain shift mechanism of Hg(II) binding to DNA /21/ and an earlier suggested first-step

binding of Hg(II) between adjacent thymine residues in one strand of DNA /6/ can induce such winding angle alterations. Moreover, crosslinks by Hg(II) should be able to produce even larger conformational changes at concentration ratios $r_t \geq 0.25$. The crosslinks could be responsible for the appearance of the nonconservative CD spectra with the large negative bands (calf thymus DNA complex, $(\epsilon_L - \epsilon_R)_{\max} = -13.8$ at 285 nm), if one assumes that all bases have binding sites for intramolecular Hg(II) crosslinks and that these are saturated at $r = 0.5$. Efforts were not attempted to discriminate between different crosslink models /7,21/ by CD measurements.

These interpretations of the CD spectra with regard to conformational changes seem to be suggestive, yet all neglect an induced Cotton effect which could arise by Hg(II) binding. On the other hand, changes in electronic transition moments of the bases occur and simply can be demonstrated by UV absorption spectra. At low values of r_t a shift of the UV transition in the absorption spectra is observed (a new maximum arises at 263 nm, Fig.4), while for larger values ($r_t \geq 0.5$) the UV absorption spectra show a new band centered at $\lambda = 278$ nm.

In contrast to our observations (Fig.4) almost unchanged UV absorption spectra are found for the B to C conformational transition of DNA induced, e.g., by increasing LiCl concentration /25/. Therefore one has to suppose that changes in the CD spectra by Hg(II) complexing may reflect at least two overlapping effects: Firstly, a change of the rotational strength due to conformational alterations and, secondly, the occurrence of a new optical active electronic transition. These two contributions have to be separated for an accurate estimation of conformational changes from CD data, which cannot be done by simple means.

The relative viscosity and the overall size (determined by light scattering measurements) of the DNA-Hg(II) complex decrease with increasing values of r_t /4/. These and other properties of the complex already led Katz to suppose a condensed form of DNA in the complex. Indeed, the strong nonconservative CD spectra of the DNA-Hg(II) complexes look like the CD spectra of the compact form of ψ -type character reported

Figure 4: UV absorption spectra of calf thymus DNA-Hg(II) complexes in 60 mM CH_3COONa , pH 6.0. $\epsilon(P)$ -apparent molar extinction coefficient, values of r_t are indicated.

for DNA in systems of organic polymer solutions /19,20/, for polylysine-DNA complexes /29,30,31/, for histone f1-DNA complexes /32/, or for dehydrated DNA in LiCl-films /33/. The amplitudes and positions of positive and negative CD maxima in all these spectra are different, but the strong nonconservative so-called ψ -character of the spectra has been attributed in all cases to a condensed form of DNA. Taking into account the different types of possible interactions, i.e. ion-polymer, polymer-polymer or dehydration effects, one cannot expect, that one and the same mechanism induces the condensed forms and these all have the same degree of compactization.

For example the compact form of DNA in organic polymer solutions /34/ gives an increase in sedimentation coefficient of 5- to 10-fold. The DNA-Hg(II) complexes have only a 3- to 4-fold larger sedimentation coefficient /5,8/, which despite of added mass of Hg(II) in the complex is distinctly larger than that of the most other DNA-metal complexes /35/.

The formation of an AT-specific Hg(II) complex followed by a GC-specific one does not contradict such a transition of DNA to a ψ -like form. As already stated a compact form of the complex arises if 1 Hg(II) per base pair is bound inducing

strong distortions of the DNA helix. On the other hand, at present no X-ray diffraction data are known which attribute the strong nonconservative CD spectrum of compact DNA forms in organic polymer solutions to another DNA conformation than to the B form /36/, although such an attribution is very controversial /33,37,38/. Whether the divalent Hg(II) ions are able to induce a DNA structure with a long range order as discussed by some authors for other systems of ψ -type character /31,33, 39,40/, or whether an aggregation is present originating specifically from Hg(II), cannot be decided by CD measurements alone. We believe it will be worth to study the suggested aspects of Hg(II) complexing in respect of ψ -like DNA forms in more detail.

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