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The interaction of rhodium(III) with DNA by circular dichroism studies. Comparative behaviour of dimethyltin(IV) and thallium(I)

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Summary

The effect of $RhCl_3$ on DNA helix conformation was studied by circular dichroism spectroscopy and compared to the behaviour of Me_2SnCl_2 and $TlNO_3$; the latter metal–DNA systems have been further studied by ultraviolet and thermal denaturation techniques. Both rhodium(III) and tin(IV) react with the bases of DNA and induce appreciable alterations of the double-helical conformation. Rhodium(III) at low binding ratio causes a stabilization of the DNA B conformation, while at higher structural changes are interpreted in terms of a B to C transition. Tin(IV) produces a ψ form geometry; only at high binding ratios does interaction with the phosphate groups seem to occur. The data for thallium(I) are interpreted in terms of a preferential interaction with the phosphate groups of the DNA chain, causing a stabilization of the double-helical structure of DNA.

Introduction

Metal ions interact with DNA in a number of distinct ways, causing different conformational changes depending on the exact mode of binding[1]. In general, interactions which stabilize the double helix, e.g. neutralization of the charged phosphate backbone, lead to an increased Tm value[2], whereas destabilizing interactions, such as base-binding and disruption of hydrogen bonding, lead to a decrease in the Tm value[3]. There is, however, no clear-cut distinction between these modes of binding, especially for metal ions, because both types of interaction may occur[4]. In this case the Tm value gives information on overall stability constants.

Rhodium and tin are among the elements whose compounds have been found to possess antitumour properties. Rhodium carboxylates exhibit carcinostatic activity[5,6] against many types of tumour, being amongst the most promising of the second generation anticancer compounds. The biological activities of organotin compounds have been extensively investigated, and in the 1980–1982 period alone more than 1200 organotin compounds were screened at the National Cancer Insititute in the USA[7]. The best results were obtained with complexed and uncomplexed diorganotin derivatives[8,9]. The effectiveness of rhodium and organotin compounds as drugs is thought to be due to their interaction with DNA[9–11].

Thallium is a cumulative poison and causes gastrointestinal and nervous system disorders[12]. Thallium carbonate is potentially mutagenic[13].

As part of our investigation on the interactions of metal ions with DNA[14,15] and in view of the great interest in this field[16], we report here the conformational changes of DNA induced by ions with promising anticancer activity such as rhodium(III), dimethyltin(IV) and thallium(I).

Experimental

Calf thymus DNA type I, highly polymerised, was obtained from Sigma; $TlNO_3$, $RhCl_3$ and Me_2SnCl_2 from Merck; AcOH from Aldrich; and AcONa from Carlo Erba.

Thermal denaturation studies

Stock solutions of 5×10^{-3} M [DNA-P] were prepared by adding 17 mg calf thymus DNA to 10 cm^3 of a 0.2 M AcONa solution (the pH was adjusted to 7.0 by the dropwise addition of AcOH) and gentle shaking at room temperature for about 48 h. The stock solution was checked spectrophotometrically at 260 nm for DNA content and kept refrigerated. Working solutions were prepared before each experiment by 1:100 dilution of the stock solution with a buffer solution AcOH/AcONa (pH 5.1) which had 8×10^{-3} M [Na^+]. The amount of metal ion added to the DNA solution was expressed as r, the molar ratio of metal to phosphorus in DNA; $r = [M^{n+}]/[\text{DNA-P}]$.

Melting curves were taken on a 551S Perkin-Elmer spectrophotometer at various metal ion/DNA ratios, r, and at various interaction times. The rate of temperature increase was 1° C/min and the acetate buffer solution was used as a blank. The absorbance and temperature values were measured automatically at the fixed wavelength of 260 nm.

Circular dichroism studies

The circular dichroism (CD) measurements were carried out with a Dichrographe Mark III-S, ISA Yvon. Molar absorptivities from absorption spectra, ε, and differential molar absorptivities between the left and right circularly polarized light channels for CD, $\Delta\varepsilon$, were calculated on basis of DNA concentration[17]. CD spectra were measured in a 1 cm cell in the 350–200 nm range. The calibration of the instrument was checked with an aqueous solution of d-10-camphorsulphonic acid. Prior to use, the d-10-camphorsulphonic acid was purified by recrystallization from hot 95% EtOH and dried to constant weight *in vacuo* at 40° C. The CD spectra were recorded at 22 and 37° C for all values of r at various times of interaction. No significant changes in the CD spectra at 22° C were observed, even for prolonged times of incubation, so in this study only the initial values at 22° C are presented (incubation time 1 h).

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U.v. spectra of complex ion solutions in both the absence and presence of DNA were obtained on a Varian Cary 14 spectrophotometer.

Results and discussion

Circular dichroism studies

Native DNA of 5×10^{-5} M phosphate concentration, after 4 h of incubation at 22° C, exhibits a positive band at 275 nm and a negative band at 243 nm (Figure 1). The values of $\Delta\varepsilon$ are + 1.4 and − 2.4, respectively; the spectrum remains the same even at 37° C after different times of incubation and is mainly 'conservative' (the sum of the rotational strength is approximately equal to zero).

Figure 2 represents the CD spectra of calf thymus DNA at different input ratios of rhodium(III) to nucleotide residues in buffered aqueous solutions. A decrease in the intensity and a red shift in the position of both bands at an r value of 1 after 96 h of incubation at 37° C were observed. These effects were more pronounced at the higher input ratios $r = 5$ or 10, where a considerable reduction in the intensities of both bands and a red shift were observed. Moreover, this reduction seems to depend on the incubation time.

Aqueous solutions of $RhCl_3$ in acetate buffer, pH = 5, have been shown[18] to be in the form of *cis* and *trans* $[RhCl_3(H_2O)_3]$ and $[RhCl_2(H_2O)_4]^+$. For $r = 1$ at 22 and 37° C (Figure 2a), as the reaction starts, the observed stabilization of the B conformation is probably due to the interaction of the charged complex cation $[RhCl_2(H_2O)_4]^+$ with the negatively charged phosphate groups. On the other hand, for $r = 5$ (Figure 2b) and $r = 10$ (Figure 2c) at room temperature a weakening of the B conformation is observed. The significant changes that are observed both in the intensity and position of the bands after the interaction of rhodium(III) with DNA (Figure 2) have been interpreted in terms of a B to C transition with unwinding of the helix, decrease of the pitch and a decrease in groove size[19] (C belongs to the B family). This type of packing has been observed also in the case of DNA existing in nucleoproteins when treated with magnesium(II)[19]. The process is also thermodynamically favourable, since it has been suggested that the B to C transition requires only a very small enthalpy change ($\Delta H^\circ = 41.9$ kJ) and a positive entropy change[20].

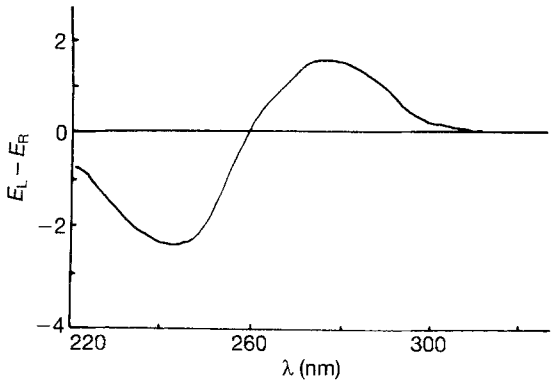

Figure 1. CD spectrum of native CT DNA 5×10^{-5} M in AcOH/AcONa 8×10^{-3} M, pH = 5.1, at 22° C after 1 h of incubation. It remains unchanged for all times of incubation at both 22 and 37° C.

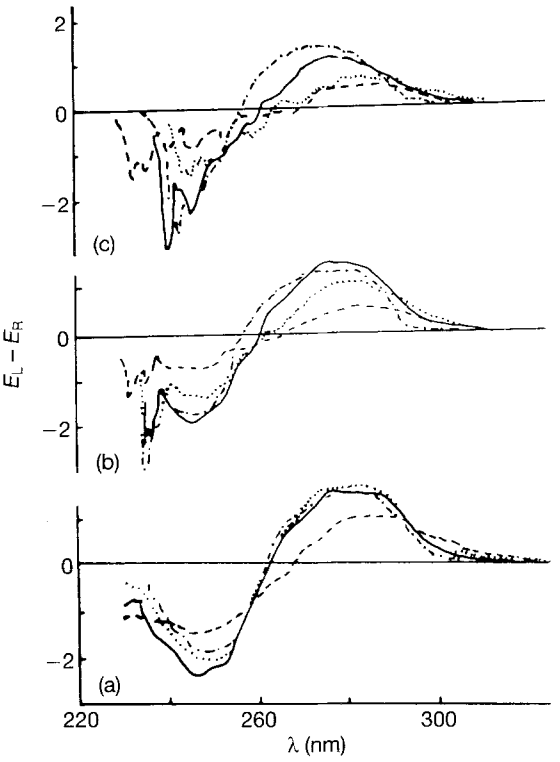

Figure 2. CD spectra of the Rh^{III}–DNA system at 37° C for different times of interaction: (——) 1 h, (····) 4 h, (– – –) 96 h, (–·–·–) 1 h at 22° C. (a) $r = 1$, (b) $r = 5$, (c) $r = 10$.

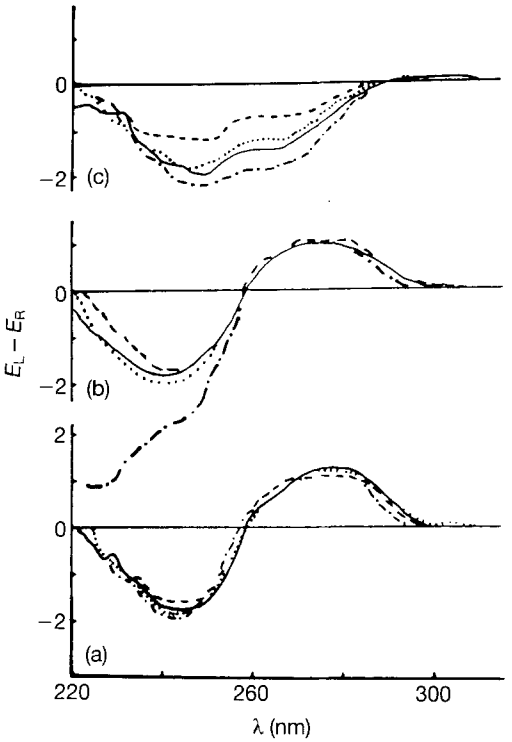

Figure 3. CD spectra of the Me_2Sn^{IV}–DNA system at 37° C for different times of incubation: (——) 1 h, (····) 4 h, (– – –) 96 h, (–·–·–) 1 h at 22° C. (a) $r = 1$, (b) $r = 5$, (c) $r = 10$.

Dimethyltin(IV) exhibits different behaviour from rhodium(III) when complexed with CT DNA. At an input ratio of $r = 10$ (Figure 3c) an inversion of the positive band is observed and the spectra of CT DNA do not maintain the original conservative character. At low binding ratios of $r = 1$ and 5 (Figure 3a and b), a

Table 1. Differential molar absorptivities between left and right circularly polarized light for CD studies of thallium(I)–DNA.

$r = 1$				$r = 5$				$r = 10$			
λ(nm)	$\Delta\varepsilon$	θ(°C)	t(h)	λ(nm)	$\Delta\varepsilon$	θ(°C)	t(h)	λ(nm)	$\Delta\varepsilon$	θ(°C)	t(h)
275	+1.2	22	1	276	+1.3	22	1	275	+1.5	22	1
244	−2.0			245	−2.0			245	−2.1		
275	+1.4	37	1	275	+1.3	37	1	275	+1.5	37	1
243	−2.1			245	−2.0			245	−2.2		
275	+1.5	37	4	275	+1.3	37	4	276	+1.4	37	4
243	−2.2			245	−2.0			246	−2.1		
276	+1.5	37	96	276	+1.2	37	96	275	+1.4	37	96
244	−2.0			244	−2.0			244	−2.2		

considerable decrease in ellipticity of the positive band is observed accompanied by a slight decrease in ellipticity of the negative band, which contrary to the rhodium(III)–DNA system, are not time-dependent. This type of CD spectrum is indicative of an intermediate conformation or a modified B form encountered during the B to Z transition[21,22]. The general shape of the distortion that appears at the high binding ratio of $r = 10$ resembles the ψ form of DNA[23]. DNA gives ψ^- spectra under the influence of MgATP[24], histone[25] and polylysine[26]. From a CD study in DNA and DNA-$(Lys)_n$ in ethanolic buffer, it has been proposed that ψ^- and ψ^+ spectra arise because of the change in the left and right coiling of the tertiary structure and have nothing to do with the secondary structure[27].

Table 1 represents the CD spectra of calf thymus DNA at different input ratios of thallium(I) to nucleotide residues in buffered aqueous solutions. A very small decrease of the positive and negative band is observed at input ratios of $r = 1$ and 5. For $r = 10$, a stabilization occurs at both 22 and 37° C for all the incubation times. The same stablization is observed for $r = 1$ after 4 h of interaction of thallium(I) with DNA. The spectra of calf thymus DNA maintain the original conservative character at any value of r and the position of the two observed bands ramains unchanged.

As a first result from our spectroscopic investigation it can be said that thallium(I) has a smaller effect on the DNA conformation than rhodium(III) and dimethyltin(IV). Its interaction with CT DNA did not alter the ordered B state, with no significant changes in DNA secondary structure or any condensation or aggregation for all the r values studied. As a matter of fact, the CD spectra (for $r = 1$ and 5) are compatible with some extension of opening and/or rotation of the stacked bases in DNA[28–30]. In the case of thallium(I), increased stability can be due either to a partial screening of the negative phosphate charges from the positively charged thallium(I) or/and to an energetically more favourable location of the complexes in the DNA major groove[31].

Thermal denaturation studies

Characteristic heating and cooling curves of DNA in the presence of the ions studied are shown in Figures 4 and 5. As shown in Table 2 the Tm values of thallium(I)/DNA solutions are higher than the Tm value of the native calf thymus DNA in the presence of 8×10^{-3} M Na^+ (Tm = 61.8° C), while most of the Tm values for the dimethyltin(IV)/DNA solutions are lower (Table 2). The

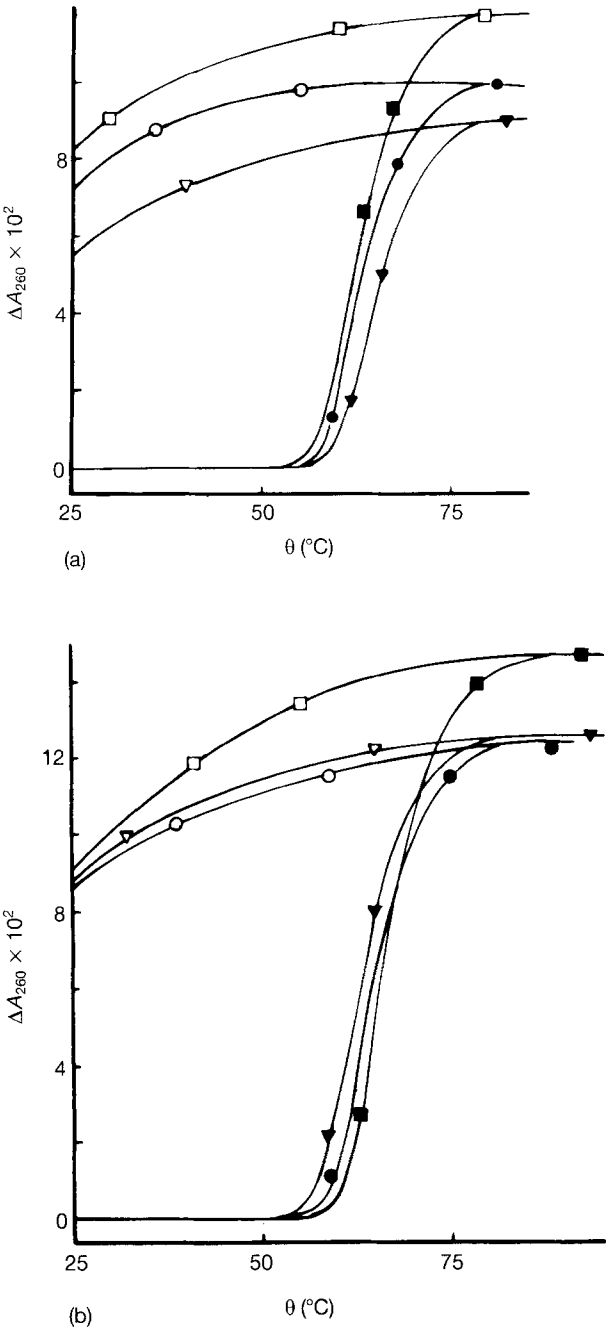

Figure 4. Melting (closed symbols) and cooling curves (open symbols) of the thallium(I)–DNA solutions. All solutions contained 5×10^{-5} M [DNA-P] in acetate buffer 8×10^{-3} M [Na^+]. Absorbances measured at 260 nm after 1 h of incubation. (a) (▼) $r = 0.5$, (■) $r = 1$, (●) $r = 2$; (b) (▼) $r = 10$, (●) $r = 20$, (■) $r = 50$.

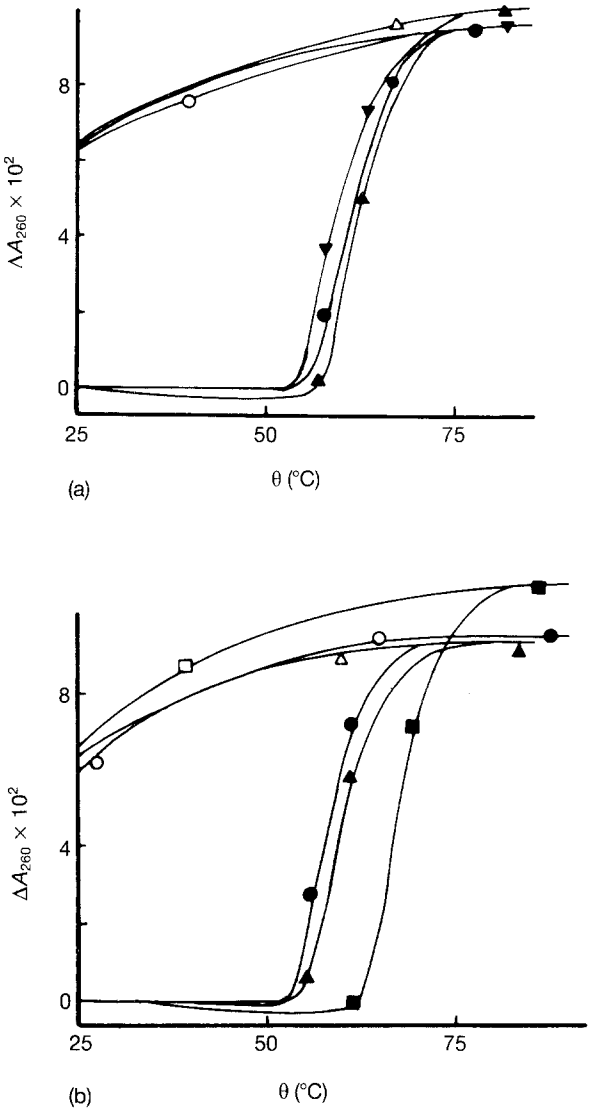

Figure 5. Thermal denaturation curves of DNA after 1 h of incubation with Me_2SnCl_2 in acetate buffer 8×10^{-3} M $[Na^+]$ solution and 5×10^{-5} M [DNA-P]. (a) (●) $r = 1$, (▲) $r = 2$, (▼) $r = 5$; (b) (▲) $r = 10$, (●) $r = 20$, (■) $r = 50$. Open symbols indicate heating curves and closed symbols cooling curves. Absorbances were measured at 260 nm.

Table 2. Melting temperatures, Tm (°C), of complex ions–DNA solutions.

t (h)	*r* = 0.5	1	2	5	10	15	20	50
$TlNO_3$								
1	64.3	62.3	62.8	62.3	62.7		64.3	65.7
24	64.8	62.2	63.8	62.7	62.9		63.7	66.8
48	64.3	63.4	63.6	63.4	64.3		62.9	66.3
Me_2SnCl_2								
1		59.9	61.5	59.6	58.3	58.6	57.2	66.7
24		59.7	61.1	59.1	58.5	58.7	58.5	66.7
48		59.9	62.0	58.3	58.6	58.8	58.0	68.0

cooling curves (Figure 4) show no significant decrease in absorbance values. The observed decrease (Figure 5) is higher in the presence of tin(IV), so in this case partial reconstruction of the denatured DNA double helix is possible upon cooling.

The interaction of $RhCl_3$ with DNA has been previously studied[16] by thermal denaturation, u.v. and viscosity measurements. Binding through both the phosphate and base moieties was suggested. At low ionic strength the melting temperature of DNA increased at low values of *r*, but decreased at higher *r* values. The DNA u.v. peak was shifted on addition of rhodium(III), indicating interaction with the bases of the DNA molecule.

In the case of thallium, from the high Tm values and the u.v. spectra, which remain unchanged after the addition of thallium, it is suggested that the aquated metal ion interacts mainly with the phosphate groups of DNA[3]. The fact that the Tm values do not increase with metal ion concentration for $r = 1$, 2, 5 and 10 also suggests a weak interaction with the bases of DNA[32]. The crystal structure of $TlNO_3$ with 1-methylcytosine[33] revealed several weak contacts to the nucleobase rather than one or two strong ones, as typically observed in nucleobase complexes of transition metal ions.

Dilute acid solutions (pH = 2) of $(CH_3)_2SnCl_2$ consist almost exclusively of the simple aquo-ion $[(CH_3)_2Sn(OH_2)_n]^{2+}$ (*n* possibly being 2 or 4[34,35]). Its hydrolysis has been extensively studied[36]. The species that dominate in dilute aqueous solutions and at pH = 5 are $[(CH_3)_2SnOH]^+$ and $(CH_3)_2Sn(OH)_2$. The environment of the metal atom in organotin(IV) moieties possibly bound to DNA has been investigated by ^{119}Sn Mossbauer spectroscopy[37,38].

The Tm values (Table 2) imply that the organotin ions interact mainly with the bases of DNA[3]. This is also supported by the observation that the u.v. maximum of DNA at 258 nm is shifted to 254 nm upon complexation with organotin ions at all ratios. At high binding ratios, interaction with the phosphate groups is suggested, leading to stabilization of the double helix.

Conclusions

From the overall spectral study, we conclude that when *cis*- and *trans*-$[RhCl_3(H_2O)_3]$ and $[RhCl_2(H_2O)_4]^+$ interact with DNA, at low binding ratio a stabilization of the double helix of DNA is observed, which may be due to neutralization of the charged phosphate backbone, while at higher binding ratios a weakening of the B conformation is observed which we interpret in terms of a B to C transition attributed to base binding and disruption of hydrogen bonding. The DNA structural changes induced by *cis*-$[Pt(NH_3)_2Cl_2]$ were interpreted as a B to C conformational transition[39,40] due to formation of intrastrand crosslinks between two DNA bases and the metal ion.

DNA under the influence of dimethyltin(IV) at high binding ratio exhibits a ψ^- spectrum. The observed weakening of the B conformation is also confirmed by the Tm experiments and base binding is suggested. Cooling leads to partial renaturation, indicative of crosslinks which thereby keep the two strands in close proximity for recombination.

The binding of thallium(I) to the B DNA conformation does not significantly alter the ordered B state, this being confirmed also by the results of the melting experiments. In this case the negatively charged phosphate backbone of the DNA helix may interact with thallium(I) either through Coulombic interactions or phosphate-oxygen binding. Neutralization of the phosphate backbone would lead to stabilization of the B conformation.

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