The dependence of *cis*-diamminedichloroplatinum(II) binding to DNA upon the GC content: a thermal and spectrophotometrical investigation

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Abstract

The binding of *cis*-diamminedichloroplatinum(II) (*cis*-DDP) to DNA is investigated as a function of the GC content and of the *cis*-DDP concentration. Analysis of the melting curves and of the UV and CD spectra shows that binding of *cis*-DDP to DNA is highly sequence specific, GC rich DNAs being destabilized more effectively. At low levels of platination, stabilization of GC–and destabilization of AT–base pairs can be noted. In AT rich DNA, *cis*-DDP binding favours the B→A conformational transition. It is also shown that, in addition to specific interactions between *cis*-DDP and DNA, several non-specific processes take place.

Introduction

DNA is believed to be the primary cellular target for the antitumour drug *cis*-DDP, and the interaction between *cis*-DDP and DNA has been investigated by many authors [1–6]. The elucidation of the mechanism of interaction of *cis*-DDP with DNA can therefore give a significant contribution to the understanding of its antitumour activity.

Several types of interactions between DNA and *cis*-DDP have been evidenced so far. It has been shown that *cis*-DDP binds covalently to cellular DNA and inhibits DNA replication [7, 8]. The *trans*-DDP isomer binds covalently to DNA as well, but it is clinically inactive and less cytotoxic than *cis*-DDP. Experiments with salmon sperm DNA have shown that the most important type of interaction of *cis*-DDP with DNA is a 1–2 intrastrand crosslink, which results from bifunctional binding of *cis*-DDP to the N(7) positions of two adjacent guanine bases in the same strand of a d(GpG) sequence, (~65% of total platination at $r_b < 0.01$) ($r_b = C_{cis\text{-DDP}}/C_{DNA}$, as number of moles of platinum bound per mole of nucleotide) [2, 9, 10]. The second type of interaction (~23%) is a 1–2 intrastrand crosslink formed at the d(ApG) sequence. The 1–2 intrastrand crosslink at the d(ApG) sequences takes place after saturation of the 1–2 intrastrand crosslink in the sequences d(GpG) at $0.02 < r_b < 0.1$ [2]. Finally a 1–3 intrastrand crosslink at the d(GpXpG) sequence (~7%) results from bifunctional attachment of *cis*-DDP to two guanines separated by any intervening base X [2, 9]. Monofunctional adducts of *cis*-DDP and interstrand crosslinks are relatively rare (~5 and 1% of total platination, respectively [11]). *cis*-DDP can also chelate at the N(7)–O(6) positions of guanine [12], although this type of interaction has never been unambiguously demonstrated. The formation of these adducts is accompanied by changes in melting temperature (T_m) and width of the melting interval (ΔT) of the helix-coil transition of DNA, and therefore, careful analysis of UV spectrophotometrical and microcalorimetrical melting curves, can give useful informations about the nature of the interactions [13, 14].

Ligands which do not interact specifically with AT or GC base-pairs, do not modify DNA melting curves and do not change the width of the helix-coil transition ΔT, but only shift the whole curve in the region of lower or higher temperatures. In the case of specific interactions between the ligands and nucleotide pairs, changes in the shape of the DNA melting curve and in the width of the melting interval take place. The

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situation is more complicated when, in addition to specific interactions, other non-specific effects take place, such as DNA breaking, formation of intra- and intermolecular aggregates, etc. [13–15].

We have suggested a simple method for investigating the specificity of the interaction of low molecular ligands with DNA base-pairs, based on the analysis of the melting curves of DNAs with different GC content [16]. From these curves the dependences of the melting temperature on GC content at different concentrations of ligands are obtained and $T_{GC}-T_{AT}$ differences are calculated by extrapolation ($X_{GC}=100\%$, $X_{GC}=0\%$). In the case of pure specific interactions a linear relationship must hold between $T_{GC}-T_{AT}$ and the width of the melting interval ΔT [17, 18]. When non-specific interactions are also present $T_m(X_{GC})$ can maintain its linear character, but the width of the melting interval ΔT will no more depend linearly on $T_{GC}-T_{AT}$. From the total ΔT, the contribution of both effects, specific and non-specific, can be separately evaluated. This method was used with success for the study of the specific interactions of metal ions with DNA [16]. In the present work the method is used for investigating the nature of the interactions of the antitumour platinum complex *cis*-DDP with DNA.

Materials and methods

Native preparations of DNA characterized by a high degree of heterogeneity were from calf thymus with exceptionally high purity (RNA < 0.1%, protein < 1%, $MW \sim 10^7$ dalton) prepared at the Institute of Bioorganic Chemistry of the Bielorussian Academy of Sciences, (Minsk, U.S.S.R.) by D. Yu. Lando. Native preparations of DNA *E. coli* (GC content = 52%, $X_{GC}=0.52$), *M. luteus* ($X_{GC}=0.72$), *Cl. perfringens* ($X_{GC}=0.28$) and polydGpolydC (pdGpdC), polydApolydT (pdApdT) were from Sigma Co. *cis*-Dichlorodiammineplatinum(II) was also from Sigma Co. Since rigorous quantitative control of the platinum complex interacting with DNA was not necessary, the concentration of the added *cis*-DDP was determined by weight. The concentration range of *cis*-DDP was 10^{-7}–10^{-4} M. Concentrations of the ligand expressed as number of moles of platinum bound per mole of nucleotide in the range $0.003 \leqslant r_b \leqslant 3$ DNA solutions in 10^{-2} M $NaClO_4 + 10^{-3}$ M NaCl were incubated with *cis*-DDP 48 h at 20 °C. The DNA concentration in UV and CD measurements, determined spectrophotometrically on a Cary 17 with thermostated cells, was 10 γ/ml. The rate of the temperature changes was 0.5 °C/min.

CD spectra were obtained on the dichrograph Jasco J 500 C. The pH of the solutions was controlled on an Orion 701/A pHmeter.

Results and discussion

In Fig. 1 the melting curves of calf thymus DNA in presence of *cis*-DDP are shown. Considerable changes of their shape in comparison with the curve of pure DNA can be observed. At low *cis*-DDP concentrations ($r_b=0.003$), destabilization of AT and stabilization of GC base-pairs can be noted. At $0.03 < r_b < 3$ destabilization of both AT and GC base-pairs and increase of the helix-coil transition width are observed.

For GC-rich DNA a different behaviour can be noted. In Fig. 2 the melting temperatures (T_m) of DNAs with different GC content (X_{GC}) at given concentrations of *cis*-DDP are reported. For all concentrations of *cis*-DDP considered, a decrease of the melting temperature is observed for *E. coli* ($X_{GC}=52\%$) and *M. lut.* ($X_{GC}=72\%$).

The changes in ΔT also depend upon the GC content (Table 1). For GC-rich DNA (*M. lut.*) ΔT increases from 4.2 ($r_b=0$) to 15.7 ($r_b=3$) °C, but for the AT-rich DNA (*Cl. perfr.*, $X_{GC}=28\%$) the increase of ΔT is only 2.0 °C. These results allow us to conclude that for concentrations of *cis*-DDP $0.003 < r_b < 3$ destabilization of both AT and GC pairs takes place, but the interaction of *cis*-DDP with DNA depends upon the GC content, GC-rich DNA being destabilized more effectively. This can be easily

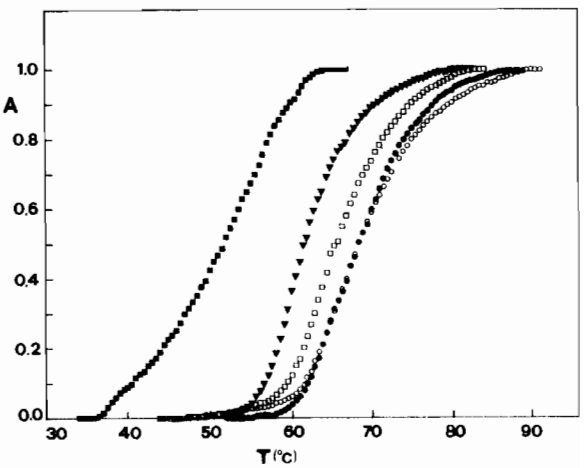

Fig. 1. Melting curves of calf thymus DNA at different concentrations of *cis*-DDP. ● Pure DNA; ○ (DNA + *cis*-DDP) $r_b=0.003$; □ $r_b=0.03$; ▲ $r_b=0.3$; ■ $r_b=3$.

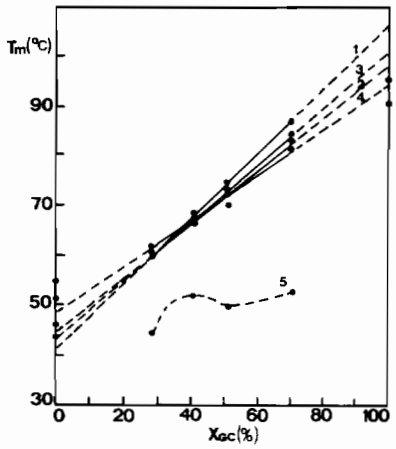

Fig. 2. Dependence of melting temperature (T_m) upon GC content (X_{GC}%) of DNA at different concentrations of *cis*-DDP. 1, Free DNA; 2, (DNA + *cis*-DDP) $r_b = 0.003$; 3, $r_b = 0.03$; 4, $r_b = 0.3$; 5, $r_b = 3$.

rationalized by considering that in GC-rich DNAs the 1–2 intrastrand crosslink interaction is more effective and the saturation of this interaction takes place at smaller concentrations of *cis*-DDP than in AT-rich DNAs. Destabilization of pdGpdC in the presence of *cis*-DDP is even more effective, and the disruption of the DNA duplex structure occurs at smaller concentrations of *cis*-DDP.

These results can be analyzed with the help of our method for the investigation of the specificity of the interaction of low-molecular ligands on nucleotide pairs of DNA [16]. It is clear from Fig. 2 that $T_m(X_{GC})$ has a linear character for $0.003 < r_b < 0.3$, which is indicative of a non-cooperative or weakly anticooperative interaction of *cis*-DDP with DNA [19]. It is necessary to point out, that T_m data for pdApdT and pdGpdC do not lie on $T_m(X_{GC})$ lines at the given concentration of *cis*-DDP (Fig. 2). This most probably can be explained by the fact that the interaction of *cis*-DDP depends upon the sequence of base-pairs of DNA, which will not be the same for DNA, pdApdT and pdGpdC.

In Table 1 the parameters $T_{GC} - T_{AT}$ and ΔT of calf thymus DNA at variable concentrations of *cis*-DDP are reported. The differences $T_{GC} - T_{AT}$ were calculated by extrapolating to $X_{GC} = 0$ and $X_{GC} = 100$ for each concentration of *cis*-DDP (Fig. 2). As mentioned above, for DNA of higher organisms characterized by a high degree of heterogeneity of the GC content, the value of the width of the transition interval should vary linearly with $T_{GC} - T_{AT}$ [17]. Linearity holds also for complexes of DNA with small ligands in case of pure specific interactions, if $T_m(X_{GC})$ maintains its linear character [18]. However, the results summarized in Table 1, indicate that the width of the temperature melting interval increases with the concentration of *cis*-DDP, whereas $T_{GC} - T_{AT}$ decreases. Thus we can state that the changes of the parameters ΔT and $T_{GC} - T_{AT}$ are not only the result of specific interactions with AT or GC pairs, but also of other factors which cause variations in relative stability of different regions of DNA, melted with different degrees of denaturation. The nature of specific and nonspecific factors can be discussed on the basis of our CD and UV data.

In Fig. 3 the UV differential spectra of the complexes *cis*-DDP–DNA are reported. The relative changes of negative and positive parts of differential UV spectra (DUVS) depend clearly upon the GC

TABLE 1. Values of T_m, ΔT and ΔH[a] of different DNAs at different concentration of *cis*-DDP[b]

Concentration of *cis*-DDP, r_b	$T_{GC} - T_{AT}$	T_m (°C)	ΔT (°C)	ΔH (%)	T_m (°C)	ΔT (°C)	ΔH (%)
		Cl. perfringens			Calf thymus		
0	64.5	58.5	5.5	33	68.0	10.0	38
0.003	53.0	60.0	6.8	32	68.5	10.3	39
0.03	56.0	58.5	7.2	26	66.7	12.0	35
0.3	45.5	58.5	7.3	23	66.0	12.6	30
3		45.0	7.5	20	51.6	13.8	13
		E. coli			*M. luteus*		
0	64.5	74.5	5.8	34	85.5	4.2	32
0.003	53.0	72.5	8.7	32	81.0	10.7	32
0.03	56.0	71.0	12.5	30	83.0	10.6	20
0.3	45.5	68.5	13.0	28	80.0	12.7	18
3		49.5	13.5	10	52.0	15.7	8

[a]Hypochromic effect, $\Delta H = \frac{A_c - A_h}{A_h} \times 100\%$, where A_c is the optical density of DNA in the coil state, A_h is the optical density of DNA in the helix state. [b]All measurements are in solutions 10^{-2} M $NaClO_4 + 10^{-3}$ M NaCl.

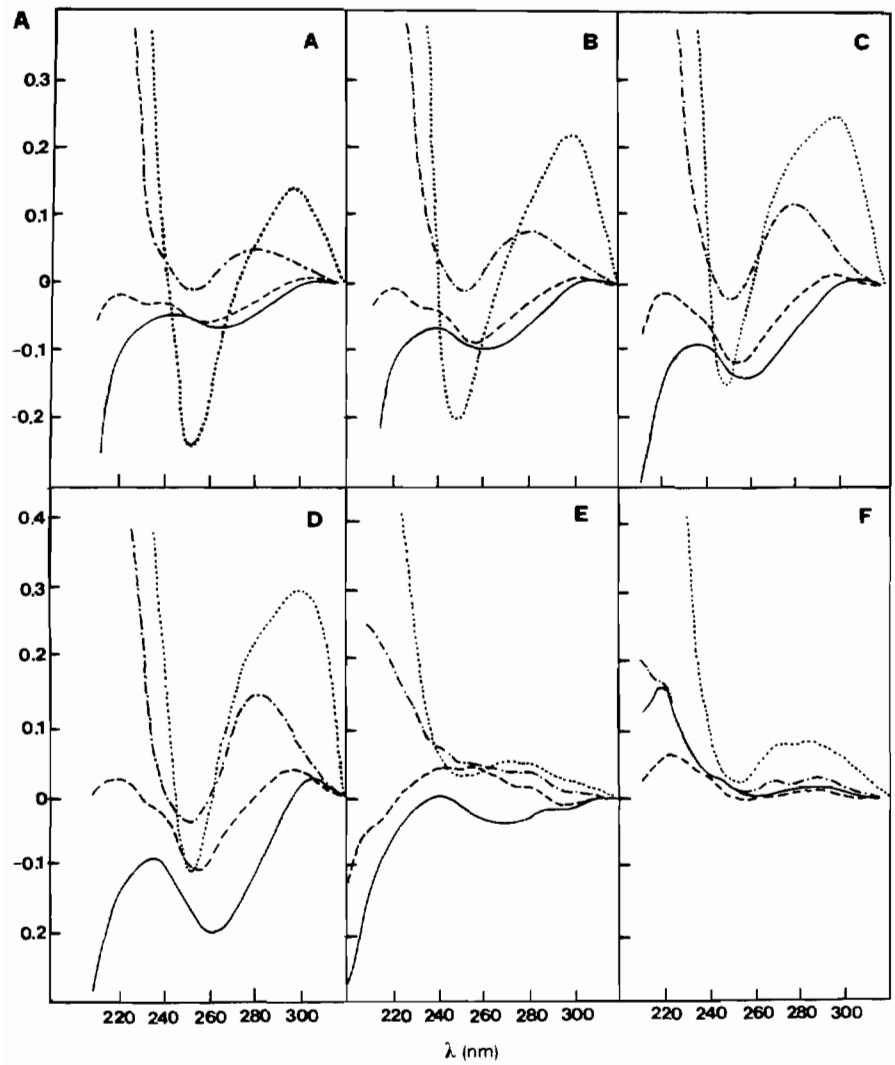

Fig. 3. UV differential spectra of the complexes DNA–*cis*-DDP at different GC contents (at 25°). A, *Cl. perfr.*, B, Calf thymus; C, *E. coli*; D, *M. lut.*, E, pdApdT; F, pdGpdC. —— $r_b = 0.003$; – – – $r_b = 0.03$; – · – · $r_b = 0.3$; · · · · $r_b = 3$.

content of DNA. For pdGpdC the negative part of DUVS is absent. The relative changes in the positive parts of DUVS are larger for GC-rich DNA (*M. lut.*) whereas changes in negative parts are larger for AT-rich DNA (*Cl. perfr.*). In the case of pdApdT and pdGpdC the changes in DUVS are not as strongly marked as in the case of DNA. This again shows that the actual mechanism of DNA–*cis*-DDP interaction depends on local sequences of base-pairs. The same conclusions can be reached by examining the CD spectra of the complexes DNA–*cis*-DDP (Figs. 4 and 5). It is clear that there is a correlation between the increase of the positive band of CD spectrum (up to $r_b \simeq 0.03$) and the GC content of DNA. The largest increase of the positive band of the CD spectrum takes place for *Cl. perfr.* DNA ($X_{GC} = 28\%$), the smallest one for *M. lut.* ($X_{GC} = 72\%$) (Fig. 5). The results of CD investigations show that in AT-rich DNA the 1–2 intrastrand crosslink formation in the sequences d(GpG) takes place at low concentration of *cis*-DDP (up to $r_b \simeq 0.03$). Probably the increase of the positive band of CD is the result of 1–2 intrastrand crosslink formation in the sequence d(ApG) as well.

The increase of the CD positive band without essential changes in the negative band at *cis*-DDP concentrations $0.003 < r_b < 0.03$ shows that a conformational transition takes place in the double-helical structure of DNA [20, 21]. It has been shown that in presence of *cis*-DDP the sugar ring conformation changes from C2′-*endo* to C3′-*endo* [4]. Since the A-form of DNA is characterized by the C3′-*endo*

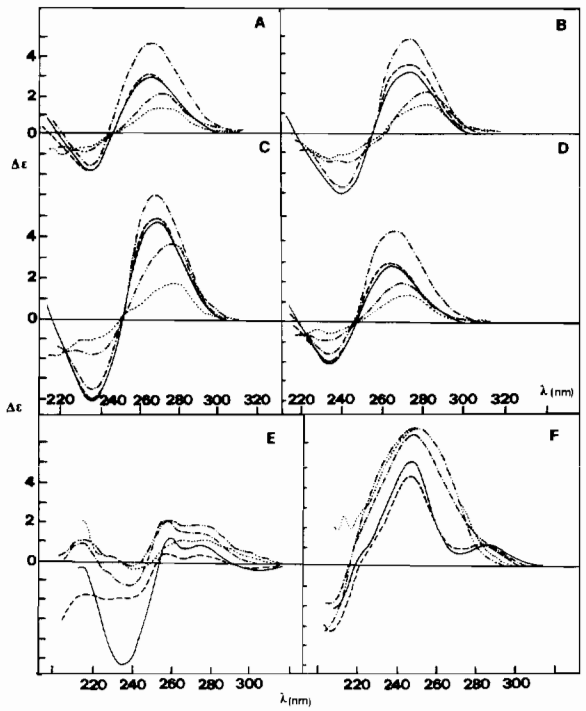

Fig. 4. CD spectra of the complexes DNA–*cis*-DDP at varying GC contents (at 25 °C). A, *Cl. perfr.*, B, Calf thymus; C, *E. coli*; *D*, *M. lut.*, E, pdApdT; F, pdGpdC. —— Free DNA, – – – $r_b = 0.003$; – · – · – $r_b = 0.03$; – · · – $r_b = 0.3$, · · · · $r_b = 3$.

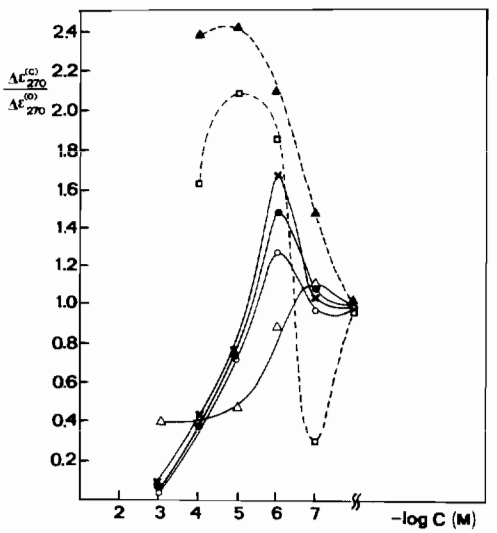

Fig. 5. Relative changes of $\Delta\epsilon_{270}$ (from CD spectrum) of DNA with different GC content and pdGpdC, pdApdT at increasing *cis*-DDP concentration (at 25 °C). Δ, *M. lut.*; ○, *E. coli*; ●, Calf thymus; ×, *Cl. perfr.*; ▲, pdGpdC; □, pdApdT.

conformation of the sugar ring, we can conclude that in AT-rich DNA, in the presence of *cis*-DDP, the conformational transition B→A will be easier than in GC-rich DNAs.

Some non-specific factors which lead to changes in conformation and thermostability of DNA have been already mentioned. The interstrand crosslink interaction is favoured by the melting process [22] and can lead to breaking down of DNA into the deoxyribonucleoside constituents. The appearance of light scattering in UV spectra of platinated DNA at 310 nm (after denaturation of DNA at $0.3 < r_b < 3$) and the essential increase of ΔT, show that formation of aggregated forms of DNA takes place as well [23, 24]. The aggregate formation, the increase of the number of interstrand crosslinks and the process of breaking down of DNA into the deoxyribonucleoside constituents lead to an increase of the width (ΔT) of the helix-coil transition interval. The increase of ΔT can also be the result of an N(7)–O(6) type of chelation of guanine bases, with formation of a strong hydrogen bond between an amine group of *cis*-DDP and O(6) of guanine [12, 25].

Thus one can conclude that our method of investigation of the interaction of low molecular ligands with AT or GC base-pairs applied to *cis*-DDP–DNA complexes can give informations not only about the specificity of the interaction, but also additional informations about non-specific effects, which can also change the character of the melting process (formation of thermodynamically more or less stable structures of DNA in presence of the ligand [19], aggregate formation [23, 24], breaking processes [22] and others).

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