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Physics

COMPARATIVE ANALYSIS OF THE EFFECTS OF PLATINUM COMPOUNDS ON THERMOSTABILITY OF DNA

I. E. GRIGORYAN*, Ye. B. DALYAN, S. G. HAROUTIUNIAN

Chair of Molecular Physics YSU, Armenia

The thermal denaturation profiles of Calf Thymus DNA in the presence of binuclear compounds of bivalent platinum (cis-[{$Pt(NH_3)_2Cl$}$_2$Pyr]Cl_2) were studied by absorption spectrophotometry and differential adiabatic scanning microcalorimetry. A comparison with an active antitumor drug cis-diammine-dichloroplatinum (cis-DDP) was made. Experimental data witness that small concentrations (r<0.3) of binuclear platinum compound stabilized the structure of Calf Thymus DNA stronger than cis-DDP. Both the complexes tend to refold the denaturated DNA. It is rather interesting that in case of 10^{-6} M concentration of binuclear platinum compound (r=0.03) the denaturated DNA is refolded by 77.62%, whereas under the same condition cis-DDP leads to 49.01% refolding of denaturated DNA. Such a high percent of renaturation was probably the result of interstrand cross-links formation.

***Keywords*:** DNA, binuclear compound of bivalent platinum, cis-DDP, renaturation, DNA melting, interstrand cross-link.

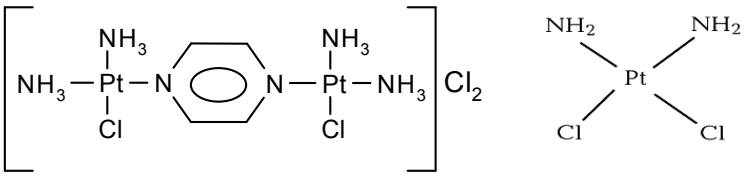
Introduction. One of the most important tasks of molecular biology is the study of the effect of biologically active ligands on the structure and properties of the DNA molecule. The secondary structure and bending rigidity of DNA may be changed as a result of interaction with biologically active compounds. The first inorganic antitumor agent, cis-diamminedichloroplatinum (cis-DDP), the activity of which is due to the formation of coordination bonds with DNA, has been widely used in the medical practice for thirty years [1, 2]. The study of molecular mechanism of its action is under way [3, 4].

It has been shown [5–8], that cis-DDP forms mainly intrastrand cross-links on DNA between neighboring purine residues (~90%), the other minor adducts being intrastrand cross-links between two purine nucleotides separated by one or more nucleotides and few of adducts remaining monofunctional. It is important that cis-DDP also forms interstrand cross-links. DNA interstrand cross-links of platinum complexes may play an very essential role in the biological activity of these compounds, because the cross-links preventing the separation of two strands of the DNA can block the DNA replication markedly more efficiently than the intrastrand adducts.

* E-mail: inessgrigoryan@yahoo.com

Even though cis-DDP is among the most successful antitumor compounds, it displays a limited activity against some of the common forms of such diseases, as the colon and breast cancers. These limitations have inspired efforts to develop new platinum based drugs that display improved therapeutic properties.

In the present work the structural changes of DNA molecule in consequence of its interaction with a new binuclear compound of bivalent platinum cis-$[Pt(NH_3)_2Cl\text{-}R\text{-}Cl(NH_3)_2Pt]Cl_2$ (bi-Pt) have been studied. A comparison with an active antitumor agent cis-DDP was made (Fig. 1).



bi-Pt cis-DDP

Fig. 1. Structural schemes of Pt(II)-containing compounds.

The binuclear platinum complex has been synthesized in the Chemical and Pharmaceutical Academy in St. Petersburg city [9].

It is well known that the interaction of cis-DDP with DNA is accompanied by coordination of cis-DDP with N7 atom of guanine [5–8]. Like cis-DDP, bi-Pt also prefers to bind N7 atom of guanine [10]. In this work we tried to clarify the type of adducts formed by bi-Pt with N7 atom of guanine as a result of interaction with DNA.

Materials and Methods. The study was performed using an ultrapure preparation of Calf Thymus DNA isolated at the laboratory headed by D.Yu. Lando at the Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus (Protein < 0.2%, RNA < 0.1%, MW ~$3\cdot10^{-7}$ *Da*, X_{GC} = 42%) [11]. All experiments have been carried out in 10^{-3} *M* NaCl+10^{-2} *M* $NaClO_4$ solution with ionic strength μ = 0.011 *M*, pH 6.4 [12].

Then DNA has been treated with bi-Pt and cis-DDP at different *mol/b.p.* ratios, and each sample was incubated at 4^0C for 48 *h*. The range of Pt(II)-containing compounds concentrations were 10^{-10}–$10^{-5}M$, or per base pair $0.000003 < r < 0.3$, where *r* is the ratio of the added Pt(II)-containing compound concentration to the concentration of DNA base pairs.

In contrast to cis-DDP the binuclear platinum complex bi-Pt under study is an electrolyte. The inner coordination sphere of the binuclear compound contains two complex-forming platinum atoms. The complex acquires positive charge upon dissociation:

$$[\{Pt(NH_3)_2Cl\}_2Pyr]Cl_2 \leftrightarrow [\{Pt(NH_3)_2Cl\}_2Pyr]^{2+} + 2Cl^-.$$

UV-absorption spectra and melting curves have been obtained with Perkin-Elmer Lambda 800 UV/VIS spectrophotometer.

The microcalorimetric measurements have been carried out with Differential Adiabatic Scanning Microcalorimeter DASM-4. The observation temperature was changed from 10 to 100^0C at the rate of $1^0C/min$. The enthalpies (ΔH) have been calculated from experimental curves of the temperature dependence of the heat capacity change (ΔC_p) using the Scal Dos software.

Results and Discussion. The studies have shown that the measurement of melting temperature (T_m) of DNA/ligand complexes is a powerful method to characterize the stability of its double helix [13].

In Fig. 2 the melting curves of bi-Pt/DNA and cis-DDP/DNA complexes at various relative concentrations ($0.0003 < r < 0.3$) of ligands (bi-Pt and cis-DDP) are shown.

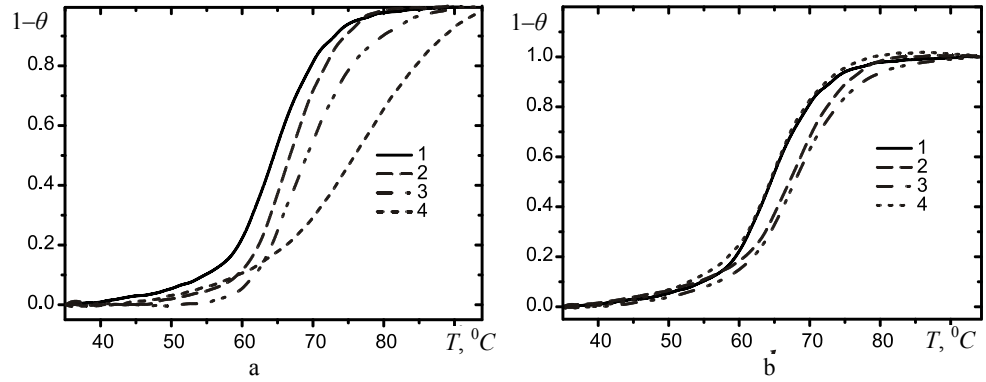


Fig. 2. Melting curves of bi-Pt/DNA (a) and cis-DDP/DNA (b) complexes in 10^{-3} *M* NaCl + $+10^{-2}$ *M* $NaClO_4$ solution: $r = 0$ (1); 0.0003 (2); 0.03 (3); 0.3 (4).

The characteristics of DNA melting at various relative bi-Pt concentrations are given in Table 1. For comparison we have calculated the parameters of melting cis-DDP/DNA complexes at various concentrations of cis-DDP under the same condition. The results are shown in Table 2.

Table 1

The values of melting parameters of bi-Pt/DNA complexes at different relative ligand concentrations

r=[bi-Pt]/[DNA]	T_m, ^0C	ΔT, ^0C	Renaturation, %
0	64.51	11.71	20.26
0.000003	66.80	12.35	22.44
0.00003	66.45	12.66	20.65
0.0003	66.69	10.96	23.18
0.003	66.35	13.22	32.52
0.006	66.36	13.23	43.98
0.012	67.00	14.06	54.88
0.018	67.04	14.20	61.78
0.025	67.85	14.85	64.94
0.03	69.09	12.69	77.62
0.06	70.95	17.35	71.52
0.13	74.82	17.67	64.08
0.3	75.91	21.05	30.36

It is apparent from Tables 1, 2 that at low concentrations $0.000003 < r < 0.03$ T_m and ΔT for both the complexes increase in comparison with pure DNA. As is seen from the Table 1, concurrent with the increase of bi-Pt concentration T_m also

increases, but in the case of cis-DDP the melting temperature reaches the maximum at $r = 0.003$ (Table 2).

Table 2

The values of melting parameters of cis-DDP/DNA complexes at different relative ligand concentrations

r =[cis-DDP]/[DNA]	T_m, ^0C	ΔT, ^0C	Renaturation, %
0	64.51	11.71	20.26
0.000003	66.29	10.98	28.17
0.00003	66.88	12.94	24.32
0.0003	67.15	14.18	29.33
0.003	69.17	13.83	34.89
0.03	67.86	13.94	49.01
0.3	64.32	13.71	24.67

In case of relatively low concentrations of ligands such a variation of parameters may be due to the cross-linking of DNA strands. To reveal the binding mechanism of DNA with Pt(II)-containing compounds the renaturation of melted complexes was also calculated. This aim in view a double melting of denaturated complexes (bi-Pt/DNA and cis-DDP/DNA) has been done. The renaturation percentage of denaturated DNA was calculated by curves of melting and double melting. The data on renaturation are given in the last columns of Tables 1 and 2. As is seen from the tables, concurrently with an increase in relative concentration ($0.000003 < r < 0.03$) both bi-Pt/DNA and cis-DDP/DNA complexes render the increase of renaturation, the highest value of renaturation for both the complexes being observed at relative concentration of $r = 0.03$. The denaturation and renaturation profiles of bi-Pt/DNA and cis-DDP/DNA complexes are shown in Fig. 3, and in case of bi-Pt/DNA complexes the renaturation is seen to be 77.62%, that is 1.5 times more than that of cis-DDP/DNA complexes.

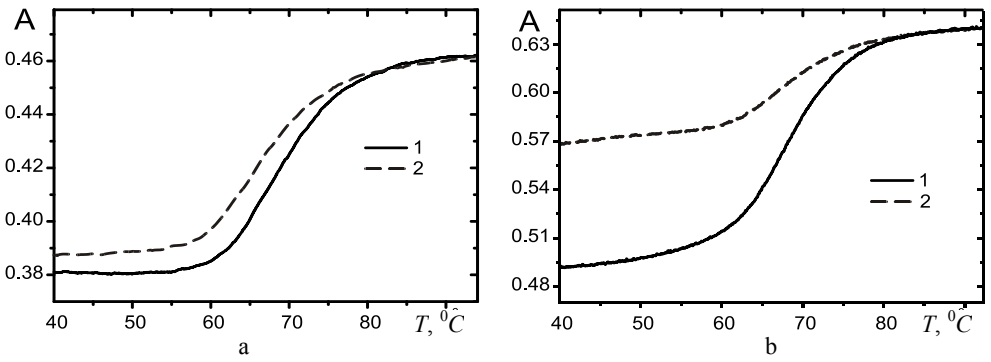


Fig. 3. Thermal denaturation (1) and renaturation (2) profiles for bi-Pt/DNA (a) and cis-DDP/ DNA (b) complexes ($r = 0.03$).

The increase of DNA renaturation after the melting attests to the formation of specific interstrand cross-links. The renaturation data obtained in case of relative concentration r=0.03 for bi-Pt/DNA as well as for cis-DDP/DNA complexes permits one to assume that an addition of Pt(II)-containing compounds

leads to the formation of interstrand cross-links, as a result of which DNA is partially refolded. Whereupon the renaturation decreases with increasing of relative concentration ($0.03 < r < 0.3$). In contrast to cis-DDP, that in case of $r > 0.03$ concentration causes single and double stranded breaks of DNA, bi-Pt forms interstrand aggregates by linking remote guanines along the chain, that is accompanied by an increase the melting temperature and the melting interval. The settling-out of bi-Pt/DNA complexes at $r = 0.3$ concentration is an evidence of that. Based on data presented in Tables 1 and 2 respectively for bi-Pt/DNA and cis-DDP/DNA complexes at $r = 0.03$ concentration, different values of enthalpy have been indirectly calculated (see Table 3).The values of enthalpy of helix-coil transition of DNA (ΔH) were determined from melting curves using an absolutely generalized formula (1) that operated with direct observational data [14] in combination with the the "area" method [15]:

$$\Delta H = \delta \frac{\delta(\Delta T)}{[\delta(T_m)]^2} \cdot T_0^2 r, \quad r << 1, \tag{1}$$

where $\delta(\Delta T) = \Delta T - \Delta T_0$, $\delta(T_m) = T_m - T_0$, T_0 and ΔT_0 being the above quantities for pure DNA.

The value of enthalpy for bi-Pt/DNA complexes as calculated by means of Eq. (1) at $r = 0.03$ concentration proved to be 4 times as small as that for cis-DDP/DNA (Table 3).

The values of enthalpy calculated from spectrophotometric curve of cis-DDP/DNA complexes at $r = 0.03$ concentration indicated that at the binding of cis-DDP to DNA there formed various types of adducts [5, 6]. The higher value of enthalpy is due to the cumulative effect of adducts caused by cis-DDP. In contrast to cis-DDP, at the binding of bi-Pt with DNA mainly the forming of interstrand cross-links are preferential. The renaturation values of these complexes (77.62 and 49.01% for bi-Pt/DNA and cis-DDP/DNA complexes respectively) additionally support of this conclusion.

In order to compare the indirect evaluation of enthalpy with its directly calculated value we have investigated the microcalorimetric melting curves of bi-Pt/DNA and cis-DDP/DNA complexes. The values of melting enthalpies were calculated from Fig. 4 and given in Table 3.

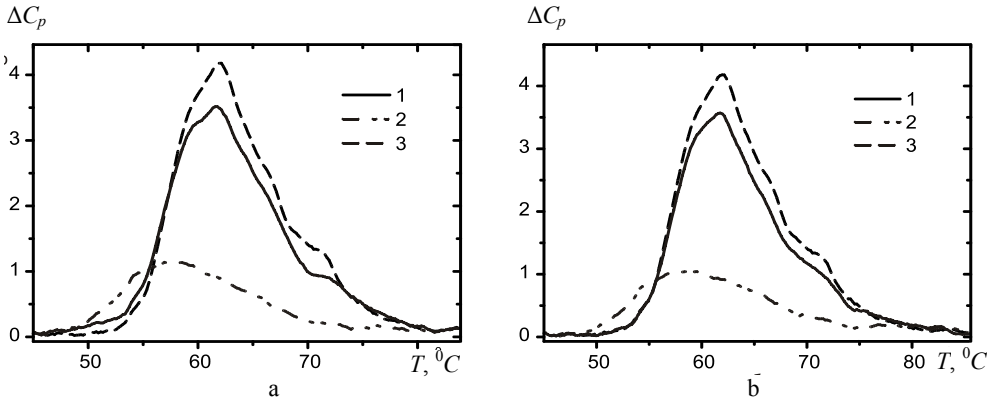


Fig. 4. Microcalorimetric melting (1) and double melting (2) curves of bi-Pt/DNA (a) and cis-DDP/DNA (b) complexes (r = 0.03) and the melting curve of pure DNA (3).

Table 3

Calorimetrically derived enthalpies for bi-Pt/DNA and cis-DDP/DNA complexes at r=0.03 relative concentration

Substance	State Condition	ΔH, *kkal/mol*	
		directly calculated	indirectly calculated
DNA	Denaturated	11.566	
bi-Pt/DNA	Denaturated	10.533	5.833
	Renaturated	4.204	
cis-DDP/DNA	Denaturated	10.138	24.808
	Renaturated	4.115	

Fig. 4 shows microcalorimetric melting curves of bi-Pt/DNA and cis-DDP/DNA complexes at r = 0.03 relative concentration, where the renaturation effect is maximal. In contrast to indirectly calculated enthalpy values of bi-Pt/DNA and cis-DDP/DNA complexes, which strongly differ one from other (5.83 and 24.81 respectively), the difference between the directly calculated values of enthalpy is insignificant (10.53 and 10.14 respectively).

It is supposed that both the Pt(II)-containing compounds show different activity towards spectral and energy properties.

Conclusion. It was shown that both the complexes stabilized the structure of Calf Thymus DNA. The macromolecule binds with bi-Pt stronger than with cis-DDP. It was supposed that the high percent of renaturation for bi-Pt/DNA complexes was the result of interstrand cross-links formation, which play very important role and bring to refolding of some DNA.

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