Comparative thermal and thermodynamic study of DNA chemically modified with antitumor drug cisplatin and its inactive analog transplatin

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ABSTRACT

Antitumor activity of cisplatin is exerted by covalent binding to DNA. For comparison, studies of cisplatin-DNA complexes often employ the very similar but inactive transplatin. In this work, thermal and thermodynamic properties of DNA complexes with these compounds were studied using differential scanning calorimetry (DSC) and computer modeling. DSC demonstrates that cisplatin decreases thermal stability (melting temperature, $T_m$) of long DNA, and transplatin increases it. At the same time, both compounds decrease the enthalpy and entropy of the helix-coil transition, and the impact of transplatin is much higher. From Pt/nucleotide molar ratio $r_b=0.001$, both compounds destroy the fine structure of DSC profile and increase the temperature melting range ($\Delta T$). For cisplatin and transplatin, the dependences $\delta T_m$ vs $r_b$ differ in sign, while $\delta \Delta T$ vs $r_b$ are positive for both compounds. The change in the parameter $\delta \Delta T$ vs $r_b$ demonstrates the GC specificity in location of DNA distortions. Our experimental results and calculations show that: 1) in contrast to [Pt(dien)Cl]Cl, monofunctional adducts formed by transplatin decrease the thermal stability of long DNA at [Na+] > 30 mM; 2) Interstrand crosslinks of cisplatin and transplatin only slightly increase $T_m$; 3) The difference in thermal stability of DNA complexes with cisplatin vs DNA complexes with transplatin mainly arise from the different thermodynamic properties of their intrastrand crosslinks. This type of crosslink appears to be responsible for the antitumor activity of cisplatin. At any [Na+] from interval 10-210 mM, cisplatin and transplatin intrastrand crosslinks give rise to destabilization and stabilization, respectively.

Keywords: DNA complexes with platinum compounds; DNA differential scanning calorimetry; DNA optical melting studies
1. Introduction

Cisplatin (cis-Pt(NH₃)₂Cl₂, Fig. 1A) is a widely used antitumor drug. Its biological activity is exerted by covalent binding to DNA [1,2]. Research on cisplatin-DNA complexes often employs the very similar but inactive transplatin (trans-Pt(NH₃)₂Cl₂, Fig. 1C) for a comparative study. In addition to transplatin, inactive diethylenetriaminechloroplatinum ([Pt(dien)Cl]Cl) is also employed in structure-activity studies. Final chemical modifications caused by cisplatin binding account for approximately 90% of intrastrand crosslinks (Fig. 1a) [3-5], 6% of interstrand crosslinks (Fig. 1b) [6-8] and a small fraction of monofunctional adducts. However, monofunctional adducts are formed in a considerable amount as intermediate products [9].

In contrast to cisplatin, transplatin monofunctional adducts (Fig. 1c) account for a considerable fraction of final products [9-13]. The averaged data of various studies give the following fractions of final adducts formed by transplatin: 35% of monofunctional adducts (Fig. 1c), 53% of intrastrand crosslinks (Fig. 1d) and 12% of interstrand crosslinks (Fig. 1e) [7,9-14]. [Pt(dien)Cl]Cl forms only monofunctional adducts that are intermediate for cisplatin. Therefore [Pt(dien)Cl]Cl is convenient for studying their properties [15]. In general, intermediate and final adducts formed by cisplatin, transplatin and [Pt(dien)Cl]Cl strongly distort the double helix [16-23]. Monofunctional adducts [24-26] and intrastrand crosslinks [27-29] decrease the melting temperature ($T_m$) at any cation concentration ($\delta T_m <0$) of short oligonucleotide duplexes of 10-20 bp.

Interstrand crosslinks formed by cisplatin and transplatin increase oligonucleotide duplex melting temperature ($\delta T_m >0$) [30, 31] because, besides local destruction, they strongly decrease the entropy of the melted state by prohibiting strand separation [32, 33]. Subtracting from the total thermal effect of the nonspecific stabilization term caused by interstrand crosslinking (or rather by a change in the molecularity of melting reaction from 2 to 1), one obtains a negative $\delta T_m$ value that corresponds to the strong local distortions at sites of interstrand crosslinking with cisplatin or transplatin [30, 31, 34]. Thus local distortions caused by all three types of modifications caused by the three considered compounds decrease thermal stability of oligonucleotide duplexes.

A special remark is necessary regarding the thermal stability of oligonucleotide duplexes that include an intrastrand crosslink formed by transplatin. In earlier studies, thermal destabilization was demonstrated [35]. However, it was shown later that this type of DNA modifications can be unstable [14]. At physiological temperature and especially under conditions of melting experiments, an intrastrand crosslink formed by transplatin transforms into the interstrand crosslink. Therefore titration calorimetry [14] has been used to measure the enthalpy and entropy of duplex formation at 4°C for association of unmodified complementary strands (coil-helix transition) as well as for an unmodified strand and a complementary strand with transplatin intrastrand crosslink that is stable at 4°C. These results [14] allowed us to assess the melting temperatures of the unmodified duplex and the duplex with intrastrand crosslink. The comparison demonstrates that the transplatin intrastrand crosslink decreases the melting temperature in the same destabilizing way as the intrastrand crosslink formed by cisplatin, although the decrease is lower. As for cisplatin, transplatin intrastrand crosslink decreases the absolute value of enthalpy and entropy of the coil-helix transition [14].

Thus, all local distortions caused by platinum compounds in the double helix usually strongly decrease the thermal stability, enthalpy and entropy of oligonucleotide duplexes. These results are in full agreement with structural data that demonstrate strong local distortions of the double helix at sites of chemical modifications [16-23]. The thermal destabilization caused by platination increases with ionic strength [27, 36]. It demonstrates that, besides structural destabilization, platinum compounds electrostatically stabilize DNA by decreasing its negative charge density. The advantage of oligonucleotide duplexes used in those studies relative to long DNAs is the identity of chemical modifications, their location and structure in each platinated duplex. However, complexes of platinum compounds with long DNAs correspond more closely to real systems, and relatively few thermodynamic investigations have been carried out with these systems.
Earlier experimental data demonstrate that, in long DNA, only cisplatin decreases $T_m$, but both transplatin and [Pt(dien)Cl]Cl increase the thermal stability ($T_m$) if melting experiments are carried out in neutral medium [36, 37]. In alkaline medium, which minimizes electrostatic stabilization, all three compounds decrease DNA thermal stability, although the decrease caused by cisplatin final products is much stronger [37]. To elucidate the mechanism of the difference in thermodynamic properties of the three platinum compounds, we have carried out comparative thermodynamic and thermal study as well as computer modeling for long chemically modified DNA. In contrast to our previous study [37], the current investigation was carried out in neutral medium, although the thermal effect of cisplatin is much higher in alkaline medium. However the DSC method can provide a more direct measurement of thermodynamic properties than UV melting used previously [37]. It allows carrying out investigation for neutral medium that is closer to real systems. Additionally using computer modeling, we have separately assessed the thermal impacts of monofunctional adducts, intrastrand crosslinks and interstrand crosslinks and explained mechanism underlying thermodynamic difference for the three platinum compounds.

2. Materials and methods

Calf Thymus DNA of Sigma-Aldrich Corporation was used. After additional purification, DNA at a concentration of 1.2 mg/mL was incubated in 10 mM NaClO$_4$ during 48 hours at 37°C in the dark at pH-6 with cisplatin or transplatin produced by the same company. DNA platination in vitro is usually carried out in NaClO$_4$ [4-6,10-13, 36], because the ion ClO$_4^−$ does not hinder platination in contrast to Cl$. For differential scanning calorimetry (DSC) measurements of platinated DNA, Pt/nucleotide molar ratio ($r$) was 0.001 to 0.05. After a 48-hour incubation, cisplatin reacts with DNA quantitatively forming mainly bifunctional adducts [36,38,39], i.e., $r=r_b$ where $r_b$ is the per nucleotide concentration of bound cisplatin.

High-resolution melting profiles of unmodified and platinated DNA were obtained using a model CSC 6300 NanoDSC differential scanning calorimeter (Calorimetry Sciences Corporation, USA) with a cell volume 0.3 mL. In the DSC experiments, we followed standard procedures [40]. For the melting studies, DNA solution was diluted to concentration 1 mg/mL and a required value of [Na$^+$] from interval 10-210 mM was adjusted with NaCl. Melting media (pH 7) also included 0.05 mM EDTA and 1 mM Na$_2$CO$_3$ that was used in our previous study [37] where alkaline medium was used for melting experiments. We have shown that replacement of 1 mM Na$_2$CO$_3$ with 5 mM cacodylate or with any other concentrations of Na$_2$CO$_3$ from 0.05 mM to 5 mM did not alter DNA melting profiles if total [Na$^+$] and pH 7 were conserved.

For UV (optical) melting studies, aliquots of the DNA-transplatin mixture ($r_b$=0.05) were withdrawn from incubation medium at time intervals 3 and 48 h. Platination in the aliquots was stopped by adjusting NaCl concentration to 100 mM. DNA solution was diluted to concentration 0.075mg/mL. Then the samples were frozen at -28°C [12]. Just before melting, DNA was thawed and diluted to 0.04 mg/mL. The optical melting was carried out in 100 mM NaCl, 5 mM Na$_2$CO$_3$, 0.05 mM EDTA, pH 7 (110 mM Na$^+$). DNA melting was recorded by measuring the optical density at 260 nm as a function of temperature ($T$) using a SF-26 spectrophotometer (LOMO, Russia).

The calorimetric differential melting curve $\vartheta'_c(T)$, corresponding melting temperature ($T_{m}$) and temperature melting range ($\Delta T_{int}$) were determined using eqs.(1)-(3):

\[
\vartheta'_c(T) = \frac{T_e}{P_{h\rightarrow c}} \int P_{h\rightarrow c}(T) \cdot dT
\]  

(1)

\[
T_{int} = \frac{\int T \cdot \vartheta'_c(T) \cdot dT}{\int \vartheta'_c(T) \cdot dT}
\]  

(2)
\[ \Delta T_{int} = \pi \cdot \frac{T_e}{T_s} \int [T - T_{int}] \cdot \theta_c(T) \cdot dT \]  

where \( P_{h-c}(T) \) is the temperature dependence of additional power applied to compensate heat absorption caused by the helix-coil transition (Fig. 2) calculated by subtraction of buffer baseline and then of sample baseline from raw data; \( T_s \) and \( T_e \) are the temperatures of the start and end of the helix-coil transition, respectively.

The advantage of the definitions of melting temperature (\( T_{int} \)) \[41\] and temperature melting range (\( \Delta T_{int} \)) given by eqs.(2)-(3) is the smoothness of their dependences on relative concentration of chemical modifications caused by platinum compounds. This issue will be discussed in detail elsewhere.

Computer modeling of the influence of chemical modifications with and without interstrand crosslinks was carried out using modified approach \[34, 42-46\] of Poland-Fixman-Friere (P-F-F) \[47, 48\]. Calculations were carried out for chemically modified homopolymeric DNA (\( GC=0\% \), \( N=5000 \text{ bp} \)), random sequence of AT and GC base pairs (\( GC=50\% \), \( N=5000 \text{ bp} \)) and EcoRI-cut pBR322 (\( GC=53.72\% \), \( N=4361 \text{ bp} \)) where \( N \) is the number of base pairs in DNA chain. For nonspecific binding, the location of \( (r_b\cdot N) \) of chemically modified base pairs was designated with a random number generator, which distributed them among all \( N \) base pairs. For the case of GC specificity of location of \( (r_b\cdot N) \) chemical modifications, they were randomly distributed among GC base pairs only, which number is equal to \( GC\cdot N/100 \).

### 3. Results and discussion

#### 3.1 A change in DSC profile under DNA chemical modification with cisplatin and transplatin

High-resolution calorimetric melting profiles (DSC curves) of the Calf Thymus DNA unmodified \((r_b=0)\) and chemically modified with cisplatin or transplatin at \( r_b=0.01, 0.025 \) and 0.05 \([\text{[Na}^+\text{]}=110 \text{ mM, pH 7}]\) after subtraction of buffer baseline and then of sample baseline \( (P_{h-c}) \) are demonstrated in Fig. 2. In unmodified DNA, the four narrow peaks enumerated from lower to higher temperatures as 1-4 are well resolved (Fig. 2). The narrow peaks stand out against a wide main peak \# 0. The narrow peaks correspond to melting of satellite DNA with quasi-periodical sequences that account for \( \sim 20\% \) of calf genome. The wide main peak \( (\sim 80\% \text{ of genome}) \) is formed by all other DNA regions with sequences far from periodicity. Satellite DNA consists of very large arrays of tandemly repeating uninterruptible sequences of \( \sim 10^6 \text{ bp} \) \[49, 50\]. For different organisms, the length of repeating units varies from one base pair to several thousand \[49, 50\]. Therefore, the melting of those satellite DNA sequences is rather similar to melting of synthetic homogeneous (poly-dA-poly-dT) or periodical (poly-dGC-poly-dGC) sequences than to simplest natural DNAs such as virulent phages or plasmids. Usually each isolated satellite sequence gives rise to a narrow peak in melting profile \[51-53\] as though poly-dA-poly-dT or poly-dGC-poly-dGC \[54, 55\] in contrast to multi-peak differential and DSC melting curves of phage and plasmid DNAs \[40, 56-58\].

DNA chemical modification with cisplatin and transplatin changes the shape and position of DSC curves. The shape is changed with \( r_b \) because of total reduction of DSC curves and gradual disappearance of four narrow peaks that form the fine structure \( (r_b \text{ is per nucleotide molar ratio of bound cisplatin or transplatin}) \). It is seen that the disappearance is caused by a stronger decrease in heights of narrow peaks relative to the main peak. To compare only the shapes of the DSC curves, they were normalized to the area to obtain the calorimetric differential melting curve \( \theta_c(T) \) (Fig. 3, Eq.(1)). The disappearance of narrow peaks is better seen for such presentation.

The beginning of elimination of narrow peaks caused by cisplatin and transplatin is registered from minimal \( r_b=0.001 \). For illustration, five normalized calorimetric melting curves for unmodified Calf Thymus DNA, three curves for DNA chemically modified with cisplatin and three curves - with transplatin were registered (Fig. 4A). Each curve corresponds to different preparation of DNA.
solution and its platination, i.e., they correspond to independent experiments. The groups of lines corresponding to unplatinated DNA and DNA modified with cisplatin or transplatin form the three wider distinguishable curves (Fig. 4A). Sometimes such approach is used in DSC studies [59]. An experimental error corresponds to the width of wider curves of each type. The negative second derivatives of the normalized DSC curves depicted in Fig. 4B demonstrate appreciable difference in melting behavior caused by this very low platination level ($r_b=0.001$). There is a 1.5- and 2-time decrease in height of the highest third narrow peak for cisplatin and transplatin, respectively (Fig. 4B).

As follows from Fig. 2-4, both cisplatin and transplatin destroy fine structure of melting curve (see also [37,56]). Transplatin causes much stronger destruction of narrow peaks and a stronger decrease in area under DSC curve than cisplatin. At $r_b=0.05$, cisplatin fully destroys the fine structure. However, the shape of the main peak changes weakly. As a result, the shape of the calorimetric differential melting curve of DNA treated with cisplatin at $r_b=0.05$ is close to the shape of the main peak of unmodified DNA.

3.2 The influence of antitumor drug cisplatin and its inactive analog transplatin on the DNA melting temperature at various ionic strengths

It is known that DNA thermal stability (melting temperature) increases with Na$^+$ concentration. The increase is much stronger for long DNAs in comparison with oligonucleotide duplexes [60]. On the other hand, all types of chemical modifications caused by platinum compounds introduce positive charge into negatively charged double helix and in this way increase DNA thermal stability. This stabilization occurs on a background of local destabilization caused by structural distortions [16-23]. As it was shown for platinated oligonucleotide duplexes, the destabilization is different for different types of modifications and for different sequences around the sites of modifications [27-29]. Therefore a general picture of a change in thermal stability of long DNAs can be very complex.

For long DNA, the dependences of the shifts of the melting temperature ($\delta T_{\text{int}}$) and of the temperature melting range ($\delta \Delta T_{\text{m}}$) on the per nucleotide molar concentration of cisplatin and transplatin ($r_b$) are shown in Fig. 5. Cisplatin decreases the DNA thermal stability and maximal destabilization is reached at highest [Na$^+$]=210 mM considered (see also [36, 37]). In contrast to cisplatin, final adducts of transplatin stabilizes DNA and maximal stabilization is reached at lowest [Na$^+$]=10 mM. That is also true for [Pt(dien)Cl]$\text{Cl}$ [36] that produces in DNA only monofunctional adducts. A common feature for the three compounds is the lowering of nonzero points ($r_b$=0) of the dependences $\delta T_{\text{int}}(r_b)$ and $\delta \Delta T_{\text{m}}(r_b)$ as [Na$^+$] is increased (Fig. 5 and [36]). For cisplatin and transplatin, the dependences $\delta T_{\text{int}}(r_b)$ are different in sign. The dependences $\delta \Delta T(r_b)$ are positive and differ only in value. However, all points except $\delta T_{\text{m}}(r_b=0)$=0 and $\delta \Delta T(r_b=0)$=0 of both dependences move down to lower temperatures for both compounds as [Na$^+$] increases. That fact demonstrates an important role of DNA electrostatic stabilization caused by platinum compounds. As follows from theory [61], the electrostatic stabilization caused by platination must be decreased with [Na$^+$], as it takes place in our case.

3.3 What type of chemical modification of transplatin conditions an increase in melting temperature of long DNA in contrast to antitumor drug cisplatin?

There was an opinion that an increase in melting temperature caused by transplatin can arise from a larger number of interstrand crosslinks and/or by lower thermal destabilization by structural distortions [36]. To study this issue we have used our previous calculations based upon the theory of helix-coil transition of DNA with interstrand crosslinks [34, 42-46]. It is known that interstrand crosslinks account for 6% and 12% of all DNA modifications for cisplatin and transplatin, respectively [6-8]. Crosslinking in itself increases the DNA melting temperature [34], but the total thermal effect of crosslinks also depends on the change in the free energy ($\delta G_{\text{el}}$) of helix-coil transition caused by local distortion of DNA structure at site of interstrand crosslink [30,34].
Our earlier calculations [34,46,62] demonstrate that a change in melting temperature (δT_m) at r_m=0.05 caused by randomly distributed interstrand crosslinks that account for 6% of all cisplatin modifications (i.e., the per nucleotide number of interstrand crosslinks r_m=0.003) can be from 0°C (for δG_{cr}=∞) to 1.8°C (for δG_{cr}=+∞). The change in melting temperature that can be caused by transplatin (r_m=0.05, 12% of interstrand crosslinks, r_m=0.006) is in the interval from 0°C (for δG_{cr}=∞) to 3.2°C (for δG_{cr}=+∞) [62]. At the same time, our experiment demonstrates that the difference in δT_m(r_m=0.05) for transplatin and cisplatin is ~10°C at [Na^+] = 10 mM and ~7°C at [Na^+] = 210 mM (Fig. 5A). Thus, a 6% higher level of interstrand crosslinking in the case of transplatin cannot explain its high stabilizing ability, because the difference in the shifts of melting temperatures caused by transplatin and cisplatin cannot exceed 3.2°C.

It is known that, during 3 hours of incubation, transplatin almost fully binds to DNA and forms monofunctional adducts [9,10]. Our earlier studies [37], in which melting was carried out in alkaline medium, demonstrated corresponding maximal decrease in thermal stability occurred just after a 3-hour incubation. Cisplatin monofunctional adducts are not stable against conditions of melting experiments in neutral medium [37], and considerable portion of them transforms into bifunctional ones before reaching DNA melting temperature (~85°C at 110 mM Na^+). For transplatin monofunctional adducts, transformation into bifunctional ones is much slower than for cisplatin [9,35,37], i.e., transplatin monofunctional adducts are more stable and can be conserved at high temperatures in contrast to monofunctional adducts of cisplatin [63].

Indeed, our UV melting experiments carried out at low time of DNA-transplatin incubation (3 h, 37°C, r_m=0.05, [Na^+] =110 mM, pH 7) demonstrate a small but reproducible decrease in melting temperature (δT_m(r_m=0.05)= -1.2±0.3°C). An approximately the same increase occurs after a 48 h incubation (δT_m(r_m=0.05)=+1.4±0.4°C). In general, it was found that monofunctional adducts of transplatin give rise to destabilization at [Na^+] > 30 mM. Close results were obtained in DSC studies. The destabilization is the same for a 3-hour incubation, although the increase in T_m for a 48-hour incubation is slightly higher. These results suggest that the stabilization caused by final adducts of transplatin mainly arises from intrastrand crosslinks, because monofunctional adducts give rise to destabilization, and interstrand crosslinking does not cause sufficient stabilization.

It is amazing how intrastrand crosslinks of transplatin increase thermal stability in contravention of stronger distortions of the double helix that are reflected in stronger destruction of fine structure of melting profile (Fig.2-4) and stronger decrease in enthalpy of the helix-coil transition (see part 3.5) relative to for cisplatin. However, it is necessary to take into account that a change in melting temperature under platination is dependent on the structural alterations of both helical and coil form of DNA. Transplatin intrastrand crosslink covers a larger region of DNA strand than cisplatin and can cause more restrictions in motion of a modified site located in melted region. The restrictions additionally decrease both the entropy of the melted state (S_e) and the entropy of the helix-to-coil transition (ΔS=S_e-S_0). Since melting temperature is equal to enthalpy-entropy ratio ΔH/ΔS, an additional decrease in entropy increases the melting temperature.

For cisplatin, the thermal impact of monofunctional and bifunctional adducts cannot be surely separated in the same way in neutral medium at low time of incubation because both intermediate monofunctional and final bifunctional adducts decrease the melting temperature, and monofunctional adducts are less stable than in the case of transplatin. Nevertheless it can be supposed that monofunctional adducts of cisplatin and transplatin are similar, and both decrease DNA thermal stability in contrast to [Pt(dien)Cl]Cl that increases the melting temperature at [Na^+] ≤ 100 mM [36,37]. The final stabilizing effect for [Pt(dien)Cl]Cl found after 48 hours of incubation [36] can be explained by higher charge introduced into the double helix by monofunctional adducts it forms.

3.4 The influence of cisplatin and transplatin on the temperature melting range of the DNA helix-coil transition
A general change in the shape of DSC melting profiles (and differential melting curves) is characterized with temperature melting range \((\Delta T_{\text{int}}\) Eq. (3)). As follows from our experimental data depicted in Fig. 5B, the dependence \(\delta\Delta T_{\text{int}}(r_b)\) is positive and increases with the degree of platination \(r_b\) at any \([Na^+]\). The increase in \(\Delta T_{\text{int}}(r_b)\) is caused by elevation of DNA heterogeneity, because a part of base pairs located in a random manner change its thermal stability under platination.

However, general reasons and our illustrative calculations (Poland-Fixman-Freire (P-F-F) method [47,48]) for the simplest case of random nonspecific distribution of modifications along poly(dA)-poly(dT) (Fig. 6) demonstrate that higher absolute value of a change in the free energy of the helix-coil transition under DNA chemical modification \((\delta G)\) must cause stronger alteration of \(T_{\text{int}}\) and \(\Delta T_{\text{int}}\). The sign of \(\delta T_{\text{int}}(r_b)\) coincides with the sign of \(\delta G\), and \(\delta\Delta T_{\text{int}}(r_b)\) is positive independently the sign of \(\delta G\).

We have also calculated the same \(\delta T_{\text{int}}(r_b)\) and \(\delta\Delta T_{\text{int}}(r_b)\) dependences as well as the dependences \(\delta T_{\text{int}}(\delta G)\) and \(\delta\Delta T_{\text{int}}(\delta G)\) for EcoRI-cut pBR322 at \(r_b=0.05\) (Fig. 7, curve "no specificity"). The value of \(\Delta T_{\text{int}}=8.5^\circ C\) for pBR322 is only slightly smaller than for the temperature melting range of the main peak in melting profile of Calf Thymus DNA \((9^\circ C)\) and can be used for modeling. It is seen from Fig. 7A (curve "no specificity") that \(\delta T_{\text{int}}(r_b=0.05)=5.5^\circ C\) for cisplatin at \([Na^+]=210\) mM corresponds to \(\delta G=\pm 1.4\) kcal per mole of modifications, but \(\delta\Delta T_{\text{int}}=0\) corresponds to \(\delta G=0\), i.e., there is no agreement. A reversed situation occurs for transplatin: \(\delta\Delta T_{\text{int}}(r_b=0.05)=\pm 1^\circ C\) corresponds to \(\delta G=0.25\) kcal and \(\delta\Delta T_{\text{int}}=2.3^\circ C\) lines with much higher value \(\delta G=1.8\) kcal. Similar contradictions take place for transplatin at \([Na^+]=10\) mM: \(\delta T_{\text{int}}(r_b=0.05)=8^\circ C\) corresponds to \(\delta G=2\) kcal, and \(\delta\Delta T_{\text{int}}(r_b=0.05)=8.9^\circ C\) corresponds to \(\delta G=3.6\) kcal.

These contradictory results are explained by selective binding of platinum compounds to GC base pairs. The value of \(\Delta T_{\text{int}}(r_b=0)=2.5^\circ C\) for the majority of natural DNAs is explained by their high thermal heterogeneity, i.e., by existing of long regions (more 100 bp) with different GC-content that are melted out at different temperature values that, in general, increase with GC because \(T_{GC}-T_{AT}>40^\circ C\). The minimal thermal heterogeneity \((\Delta T_{\text{int}}=2.5^\circ C)\) corresponds to genomes of virulent bacteriophages [57]. The same value was obtained in computer modeling for sequences formed by random distribution of AT- and GC-base pairs [57]. DNA thermal heterogeneity (and \(\Delta T_{\text{int}}\)) is additionally increased by any chemical unselective modifications that equally change thermal stability independently of the type of base pair, i.e., the binding with both AT and GC base pairs occurs and \(\delta G\) is the same for them (Fig. 7B, curve "no specificity"). However, selectivity in modification of GC base pairs stronger increases \(\Delta T_{\text{int}}\) if the modification increases thermal stability as in the case of transplatin (Fig. 7B, curve "GC-specificity", \(\delta G=0\)). If such GC selective modification decreases the thermal stability like cisplatin, then the increase in \(\Delta T_{\text{int}}\) is lower than without GC-selectivity (Fig. 7B, curve "GC-specificity", \(\delta G<0\)). Such melting behavior is seen in our experiments (Fig. 5A and 5B): besides the influence of growth of the degree of thermal heterogeneity by chemical modifications additional alteration of \(\Delta T_{\text{int}}\) takes place in agreement with a change in \(T_{\text{int}}\) and GC specificity. At same time, the GC-specificity does not influence the melting temperature and both dependences \(\delta T_{\text{int}}(\delta G)\) completely coincide (Fig. 7A). Moreover, the dependence \(\delta T_{\text{int}}(\delta G)\) at \(r_b=0.05\) calculated for poly(dA)-poly(dT) coincide with the coinciding dependences for GC-specific and unspecific chemical modification of EcoRI-cut pBR322 (Fig. 7A).

Our experiment has directly demonstrated selective stabilization of GC-rich regions of DNA by transplatin at very low level of platination and low ionic strength \((r_b=0.001, [Na^+]=10\) mM) when all narrow peaks corresponding to long satellite DNA sequences with different GC-content are well seen in DSC curves. As follows from Fig. 8, high temperature peaks corresponding to higher GC are shifted more strongly toward higher temperatures. For the first and forth narrow peaks the shift is \(0.2^\circ C\) and \(0.9^\circ C\), respectively.

3.5 The influence of cisplatin and transplatin on the enthalpy and entropy of the helix-coil transition
There is a strong decrease in the enthalpy and entropy of the helix-coil transition under platination (Fig. 9, see also [64]). For cisplatin at $r_b=0.05$, a change in enthalpy is 2.6 kcal per mole of base pairs that is equivalent to 26 kcal per mole of modifications. For transplatin, the corresponding changes are 3.8 kcal per mole of base pairs and 38 kcal per mole of modifications, respectively.

These data correspond to strong distortions of the double helix under platination with both compounds. Usually, heavy distortions cause a decrease in enthalpy, entropy and melting temperature, disappearance of fine structure of differential melting curves and an increase in temperature melting range. Ultrasonication or repeated heating-cooling of plasmid DNA solution as well as introduction of non-complementary (mismatched) base pairs give rise to such effects [58, 65].

3.6 On the role of electrostatic interactions in thermodynamic properties of DNA complexes with platinum compounds

The data of previous studies described in Introduction demonstrate that all three modifications destroy DNA structure [16–23]. Those destructions decrease the melting temperature of short DNAs (oligonucleotide duplexes) [15, 24–31]. The situation is different for long DNAs [36, 37] characterized by much stronger electrostastics. If melting is carried out at pH=7, then only cisplatin decreases the melting temperature. The decrease in the melting temperature (curve $\delta T_{m_{0}}(r_b)$, Fig. 5A) becomes two times steeper as concentration of sodium ions changes from 10 to 210 mM (Fig. 5A). It demonstrates that the thermal effect of structural distortions exceed the stabilizing electrostatic influence at any [Na$^+$], although the electrostatic stabilizing impact is prominent (see also [36]).

The positive charge introduced by platinum compounds into the double helix stabilizes DNA because the linear charge density decreases at sites of modification and around them. Such sites of stabilization cause a general positive shift relative to melting curve of unplatinated DNA. The same sites contain structural distortions that shift the melting curves to lower temperature.

Thus the electrostatic stabilization hides the influence of structural distortions on DNA thermal stability especially at low ionic strength. For [Pt(dien)Cl]Cl that forms only monofunctional adducts and for final adducts of transplatin, the thermal impact of electrostatic stabilization is even higher than the structural destabilization (Fig. 5A and [34, 36]). Transplatin final adducts increase DNA thermal stability at any [Na$^+$] from 10 to 210 mM. The stabilization caused by final adducts is maximal at low ionic strength. In contrast to [Pt(dien)Cl]Cl monofunctional adducts, transplatin monofunctional adducts cause destabilization at [Na$^+$]>30 mM.

3.7 Mechanism of the disappearance of the fine structure in melting profiles

For primary ($P_{b,c}(T)$, Fig. 2) and normalized ($\delta'_{c,r}(T)$, Fig. 3, Eq.(1)) melting profiles of unmodified DNA ($r_b=0$), the area of the $i$-th peak ($S_i$) is proportional to the product of its height ($h_i(0)$) and temperature melting range ($\Delta T_i(0)$):

$$S_i(0) = k \cdot h_i(0) \cdot \Delta T_i(0) \quad (4)$$

where $k$ is the proportionality coefficient that depends on the shape of the peak and on the way of determination of the temperature melting range (see [32,33,66] as an example).

The relative area of each peak is approximately equal to the fraction of base pairs that originates the peak under DNA melting. Under platination, an increase in the temperature melting range ($\Delta T_i$) of a peak with conserving its relative area decreases its height ($A_i$) because

$$S_i(r_b)=S_i(0) \quad (5)$$

and
\[ A_i(r_b) = A_i(0) \times \Delta T_i(0) / \Delta T_i(r_b) \quad (6) \]

Let us suppose that, at a given \( r_b' \), the main peak (\( i=0 \)) and four narrow peaks (\( i=1,4 \)) of Calf Thymus DNA (\( \Delta T_m = 0-4 \)) increase the temperature melting range under platination by the same value \( \delta \Delta T(r_b') = 1^\circ C \) (Fig. 2 and 3). As follows from Eq. (6), for the narrowest third peak (\( T_s = 93^\circ C, \Delta T_s(0) = 1^\circ C, \Delta T_s(r_b') = 2^\circ C \)), such a change in \( \Delta T \) reduces the height \( A_3 \) by one half (Eq. (6)). For the same \( \delta \Delta T = 1^\circ C \), there is only an approximately 10%-increase in melting range and the same decrease in the height of the main peak with \( \Delta T_0(0) = 9^\circ C \). As a result of a much stronger decrease in height, the narrowest third peak becomes badly resolved in the melting profile. The same concerns other three narrow peaks, although the decrease in relative height is less pronounced for them because of larger value of \( \Delta T(0) \) (\( i=1,2,4 \)). It explains the disappearance of fine structure formed by narrow peaks in the melting profiles. However, a remark on our unobvious condition of \( \delta \Delta T_i \) equality at a given \( r_b \) is necessary.

It is known that the temperature melting range overlapped by melting curves increases with the degree of DNA thermal heterogeneity [66]. The thermal heterogeneity is determined by the existence of DNA regions longer 100 bp that are different in GC-content and melted out at different temperatures increased with GC. For DNAs shorter 100 kbp, such regions originate fine multipack structure of melting profiles. Higher heterogeneity implies higher average difference (dispersion) in GC-content for these regions and consequent higher value of \( \Delta T_{im}(r_b=0) \).

Our computer modeling using the P-F-F method demonstrates that a positive change in the temperature melting range (\( \delta \Delta T_{im}(r_b) \)) caused by randomly distributed chemical modifications decreases in value with DNA thermal heterogeneity (with \( \Delta T_{im}(r_b=0) \)). It means that for homopolymers, periodical and quasi-periodical satellite sequences, \( \delta \Delta T_{im}(r_b) \) is higher than for more heterogeneous sequences such as pBR322 (\( \Delta T_{im} = 8.5^\circ C \)), although \( \Delta T_{im}(r_b=0) \) is smaller for the less heterogeneous sequences. It means that a smaller increase relative to peaks of satellite DNA must take place for the main (zero) peak in the melting profile of Calf Thymus DNA. For the main peak, \( \Delta T_0 = 9.0^\circ C \) is very close to \( \Delta T_{im} = 8.5^\circ C \) of the plasmid DNA used in calculation. It means that the width of narrow peaks # 1-4 corresponding to satellite DNA increases and the height decreases more strongly under platination than for the main much wider peak.

For simplification of our consideration using Eq. (6), we supposed \( \delta \Delta T(r_b') \) to be equal for all peaks with numbers 0-4. Our computer modeling using P-F-F method demonstrates a stronger relative decrease in height of narrow peaks # 1-4 (Fig. 2) than for the main band (#0). Therefore real elimination of fine structure in melting profiles under platination is even stronger than it is predicted by Eq. (6).

It is known that the fine structure of plasmid DNA also eliminates under platination [56]. However, the mechanism of elimination is different and will be considered elsewhere.

4. Conclusion

Experimental results of this work demonstrate a decrease in the enthalpy and entropy of helix-coil transition of long DNA caused by cisplatin and transplatin final adducts. That is in agreement with strong local structural distortions they cause in the double helix and with previous results obtained for short DNAs (oligonucleotide duplexes). Considered platinum compounds decrease melting temperature of short DNAs. That also corresponds to the structural destructions. However it is not true for complexes of long DNAs with transplatin that increases the melting temperature. At low concentration of cations, the increase is very strong. Those experimental results contradict the data for distortions caused by other damaging factors.

Computer modeling and additional experiments for low incubation time of DNA with transplatin demonstrate: 1) monofunctional adducts of transplatin decrease the thermal stability; 2) the calculated increase in thermal stability caused by interstrand crosslinks is too small to explain the
experiment. These results allow us to conclude that the difference between thermal stabilities of DNA complexes with cisplatin and transplatin mainly arises from different thermodynamic properties of their intrastrand crosslinks that are responsible for antitumor activity of cisplatin. The conclusion is in agreement with our previous melting studies carried out in alkaline medium [37].

Both cisplatin and transplatin increase the temperature melting range and destroy fine structure of calorimetric melting profiles that is in agreement with the action of other destructive factors. Short DNAs do not exhibit the fine structure in their DSC curves. Therefore, the comparison cannot be carried out for this character of DNA melting.

Using computer modeling, we have investigated different mechanisms that potentially can change the shape of DSC melting profiles. It was found that a change in the shape of melting curves under platination can be explained by the joint influence of an increase in DNA heterogeneity under platination and selective binding of platinum compounds to guanine. The selective stabilization of GC-base pairs by transplatin additionally increases the temperature melting range. At the same time, selective destabilizing of GC-base pairs by cisplatin decreases this parameter.

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References

**Figure Captions**

**Fig. 1.**  A) Cisplatin. B) DNA chemical modification with cisplatin: intrastrand crosslink between two neighboring guanines or between guanine and adenine of the same DNA strand (a), and interstrand crosslink between two neighboring guanines of different DNA strands (b). C) Transplatin. D) DNA chemical modification with transplatin: monofunctional adduct at guanine (c), intrastrand crosslink between two guanines separated with a base of any type (d), and interstrand crosslink between guanine and cytosine of the same base pair (e).

**Fig. 2.** The high resolution calorimetric melting profile for Calf Thymus DNA before \((r_b=0)\) and after \((r_b=0.01-0.05)\) its chemical modification with cisplatin (A) and transplatin (B). The four narrow peaks are marked with integers 1-4. The values of the molar Pt / nucleotide ratio \(r_b\) are shown with decimals. Melting was carried out in 108 mM NaCl, 0.05 mM EDTA, 1 mM Na₂CO₃, pH 7. DNA concentration is 1 mg/mL. The curves were calculated by subtraction of buffer baseline and sample baseline from raw data.

**Fig. 3.** A) The melting profiles from Fig. 2 normalized to their area to obtain calorimetric differential melting curve \(\Delta f(T)\). \(r_b\) values are shown in the figure.

**Fig. 4.** A) The calorimetric melting profiles normalized to area obtained before (5 curves) and after DNA chemical modification with cisplatin (3 curves) and transplatin (3 curves) at per nucleotide concentration \(r_b=0.001\). B) The negative second derivatives of the DSC curves depicted in Fig. 4A. The melting conditions are described in the legend to Fig. 2. Each of the three groups of curves forms three wider curves seen in the Fig. 4A and 4B. An error corresponds to the width of the curve of each type.

**Fig. 5.** The dependences of a change in the melting temperature \((\Delta T_m)\) (A) and in temperature melting range \((\Delta \Delta T_m)\) (B) on the per nucleotide concentration \((r_b)\) of bound cisplatin and transplatin. Melting was carried out at various [Na⁺]. The dependences correspondent to 10 and 210 mM are shown in the figure. The curves for [Na⁺]=30, 60, 100, 110 mM are located in falling manner between the curves 10 and 210 mM. Error bars are shown when they exceed the size of the marks of experimental points.

**Fig. 6.** Calculated \(r_b\) dependences of a change in melting temperature (A) and temperature melting range (B) caused by randomly distributed chemical modifications that change the free energy of helix-coil transition at the sites of their location by \(\Delta G\). Calculation was carried out for poly(dA)-poly(dT). \(\Delta G\) values are shown in the figure (kilocalories per mole of modifications).

**Fig. 7.** Calculated dependences of a change in melting temperature (A) and temperature melting range (B) caused by randomly distributed chemical modifications on a change in the free energy of helix-coil transition \((\Delta G)\) at the sites of modification for \(r_b=0.05\). Unspecific random distribution among all AT and GC base pairs ("no specificity", circles) and specific distribution among only GC base pairs ("GC-specificity", squares) are considered. Calculation was carried out for EcoRI-cut pBR322. For GC-specific and unspecific binding, the dependences \(\Delta T_m(r_b)\) coincide and \((\Delta \Delta T_m)\) are different.

**Fig. 8.** Illustration of GC-specificity of DNA stabilization by transplatin at very low per nucleotide concentration \(r_b=0.001\) using the negative second derivatives of calorimetric melting profiles obtained before and after DNA chemical modification with transplatin. High temperature peaks corresponding to DNA regions of higher GC-content demonstrate stronger positive shift. Melting was carried out at [Na⁺]=10 mM, pH 7.
Fig. 9. The dependences of the helix-coil transition enthalpy ($\Delta H$) and entropy ($\Delta S$) on the relative per nucleotide concentration ($r_b$) of bound cisplatin and transplatin. The results of three independent experiments carried out for each compound are shown in the figure. The conditions are described in the legend to Fig. 2.
Synopsis for the Graphical Abstract

Cisplatin and transplatin decrease enthalpy of DNA helix-coil transition by 26 and 38 kcal/mole per modifications. The fine structure of melting profile disappears from Pt/nucleotide=0.001 to 0.05. In contrast to antitumor drug cisplatin, intrastrand crosslinks of inactive transplatin increase DNA thermal stability. Monofunctional adducts of transplatin decrease DNA thermal stability.

Highlights

> Cisplatin and transplatin decrease enthalpy and entropy of DNA helix-coil transition
> The fine structure of DNA thermogram gradually disappears from Pt/nucleotide=0.001
> Intrastrand crosslinks of cisplatin (active) decrease DNA thermal stability
> Intrastrand crosslinks of transplatin (inactive) increase DNA thermal stability
> Monofunctional adducts of transplatin decrease DNA thermal stability
Fig. 1.  A) Cisplatin. B) DNA chemical modification with cisplatin: intrastrand crosslink between two neighboring guanines or between guanine and adenine of the same DNA strand (a), and interstrand crosslink between two neighboring guanines of different DNA strands (b).

C) Transplatin. D) DNA chemical modification with transplatin: monofunctional adduct at guanine (c), intrastrand crosslink between two guanines separated with a base of any type (d), and interstrand crosslink between guanine and cytosine of the same base pair (e).
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Fig. 3. A) The melting profiles from Fig. 2 normalized to their area to obtain calorimetric differential melting curve $\theta_cT(T)$. $r_b$ values are shown in the figure.
Fig. 4. A) The calorimetric melting profiles normalized to area obtained before (5 curves) and after DNA chemical modification with cisplatin (3 curves) and transplatin (3 curves) at per nucleotide concentration $r_b=0.001$. B) The negative second derivatives of the DSC curves depicted in Fig. 4A. The melting conditions are described in the legend to Fig. 2. Each of the three groups of curves forms three wider curves seen in the Fig. 4A and 4B. An error corresponds to the width of the curve of each type.
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Fig. 7. Calculated dependences of a change in melting temperature (A) and temperature melting range (B) caused by randomly distributed chemical modifications on a change in the free energy of helix-coil transition (ΔG) at the sites of modification for $r_b=0.05$. Unspecific random distribution among all AT and GC base pairs ("no specificity", circles) and specific distribution among only GC base pairs ("GC-specificity", squares) are considered. Calculation was carried out for EcoRI-cut pBR322. For GC-specific and unspecific binding, the dependences $\delta T_{\text{int}}(r_b)$ coincide and $(\delta\Delta T_{\text{int}})$ are different.
Fig. 8. Illustration of GC-specificity of DNA stabilization by transplatin at very low per nucleotide concentration $r_b=0.001$ using the negative second derivatives of calorimetric melting profiles obtained before and after DNA chemical modification with transplatin. High temperature peaks corresponding to DNA regions of higher GC-content demonstrate stronger positive shift. Melting was carried out at [Na$^+$]=10 mM, pH 7.
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Graphical abstract

The negative second derivatives of normalized calorimetric melting profiles obtained before and after DNA treatment with transplatin.

Transplatin

$T, ^\circ C$

$r_b = 0$

$r_b = 0.001$