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The effect of ionic strength on melting of DNA modified by platinum(II) complexes

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Abstract

Thermal denaturation of calf thymus DNA modified by antitumor *cis*-diamminedichloroplatinum(II) (*cis*-DDP) and by two related Pt(II) compounds which had been shown to be clinically ineffective, viz. *trans*-diamminedichloroplatinum(II) (*trans*-DDP) or monodentate diethylenetriaminechloroplatinum(II) chloride {[Pt(dien)Cl)]Cl}, was studied by monitoring changes of absorbance at 260 nm. The melting of DNA platinated to different levels was investigated in neutral media containing varying concentrations of Na^+. It has been shown that the ionic strength has a strong influence on the character and magnitude of changes in the melting temperature of DNA (T_m) induced by the platination. The modification of DNA by either platinum complex used in this work results in an increase of T_m if DNA melting is measured in media containing low Na^+ concentrations (ca. 1 mM). This effect is reversed at higher Na^+ concentrations. The concentration of Na^+ at which this reversal occurs is, however, markedly lower for DNA modified by *cis*-DDP than for DNA modified by the other two platinum complexes. These results have been interpreted to mean that at least three factors affect the thermal stability of DNA modified by the platinum(II) complexes: stabilization effects of the positive charge on the platinum moiety and of interstrand cross-links, and a destabilization effect of conformational distortions in DNA. Thus, in order to compare and interpret the melting behavior of DNA modified by different compounds, a great attention has to be paid to the composition of the medium in which the melting experiments are carried out.

Keywords: Platinum antitumor complexes; DNA modification; DNA thermal denaturation; Structure–activity relationship; DNA stability

Biological activity of a number of low-molecular-mass compounds is derived from their interaction with DNA. Therefore, investigation of physical characteristics of DNA modified by the low-molecular-mass compounds is an important part of studies of the mechanism of their biological effectiveness. Stability of DNA double helix is one of the most frequently studied physical characteristic of DNA, which can be radically affected by its interaction with these compounds. Numerous studies have shown that the measurement of melting temperature

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Abbreviations: Bp, base pair; *cis*-DDP, *cis*-diamminedichloroplatinum(II); d(ACG), synthetic oligodeoxyribonucleotide of the base sequence of 5′-d(GGAAGAGACGGAGGA); d(CGT), synthetic oligodeoxyribonucleotide of the base sequence of 5′-d(CTCCTCCGTCTCTTC); d(CGT)/d(ACG), the duplex composed of d(CGT) and d(ACG); [Pt(dien)Cl)]Cl, diethylenetriaminechloroplatinum(II) chloride; r_b, the number of platinum atoms coordinated to DNA per one nucleotide residue; T_m, melting temperature; ΔT_m, the difference between the T_m values of nonmodified and platinated DNAs; *trans*-DDP, *trans*-diamminedichloroplatinum(II)

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of DNA (T_m) is a powerful method to study the stability of its double helix.

Several platinum coordination complexes exhibit anticancer effectiveness [1,2]. There is a large body of experimental evidence indicating that DNA is the critical target for their cytostatic activity (for general reviews, see Refs. [3–6]). As a result, interactions of platinum complexes with DNA are at present extensively investigated. *cis*-Diamminedichloroplatinum(II) (*cis*-DDP) is the first platinum drug introduced in the clinic. Structure–activity studies often compare biological and biochemical properties of *cis*-DDP with those of inactive platinum complexes. In this approach, a search for differences between active and inactive compounds which may be responsible for the pharmacological effect have been made. Among the inactive compounds an isomer of *cis*-DDP, *trans*-diamminedichloroplatinum(II) (*trans*-DDP), has been widely used to investigate the mechanism of action of the platinum complexes.

cis- and *trans*-DDP are bifunctional complexes with two leaving ligands represented by chloride groups. Both complexes react with DNA after hydrolysis replacing one or both chlorides with water [7]. In the first step, they form monofunctional adducts at the N7 position of guanine residues. In the second step, the monofunctional adducts close to various bifunctional lesions. *cis*-DDP forms on natural and linear double-stranded DNA approximately 90% intrastrand adducts between neighboring purine nucleosides, interstrand cross-links, intrastrand cross-links between nonadjacent nucleosides and monofunctional adducts amount to 10% [8,9]. *trans*-DDP forms on DNA mainly monofunctional adducts, interstrand cross-links and intrastrand cross-links between nonadjacent nucleoside residues [7,8].

The studies of pharmacological structure–activity relationships of platinum complexes also employ another inactive compound, diethylenetriaminechloroplatinum(II) chloride {[Pt(dien)Cl)]Cl} [9]. This monodentate complex is used to study the first monofunctional binding step of the bifunctional reactions of *cis*- or *trans*-DDP with DNA or monofunctional DNA adducts of these complexes which do not close to bifunctional DNA lesions.

[Pt(dien)Cl]Cl preferentially coordinates to DNA at its deoxyriboguanosine residues at their N7 position [10]. Important feature of the bifunctional coordination of *cis*- and *trans*-DDP or monofunctional coordination of [Pt(dien)Cl)]Cl to DNA is introduction of positive charges to the DNA molecule. Also importantly, the formation of the monofunctional and bifunctional adducts of the three platinum(II) complexes results in a number of different conformational alterations in the double-helical DNA.

Natural DNAs globally modified by the platinum complexes or synthetic oligonucleotide duplexes containing a single, defined adduct of the platinum complex have been already extensively characterized by the techniques of molecular biophysics, including the measurements of DNA melting temperature.

Recently the melting behavior of short oligonucleotide duplexes 9–20 base pairs (bp) long which contained a single monofunctional adduct of [Pt(dien)Cl]Cl formed at the deoxyriboguanosine residue was described [11,12]. It was found that the formation of this monofunctional adduct decreased T_m of these duplexes by 1–16°C depending on the base sequence, the length of the duplex and the ionic strength of the medium in which the oligonucleotide duplex was dissolved. This observation was somewhat surprising since the melting experiments carried out previously [9,13] with high-molecular-mass DNAs (calf thymus, salmon sperm) globally modified by [Pt(dien)Cl]Cl revealed an increase of T_m by 1–3°C at r_b values in the range of 0.01–0.1 (r_b is defined as the number of platinum atoms coordinated to DNA per one nucleotide residue). This difference has not been satisfactorily explained so far. Interestingly, bifunctional *cis*-DDP and its *trans* isomer affected T_m of high-molecular-mass DNA in different manner [9,13]. Whereas modification of DNA by *cis*-DDP resulted in a decrease of the melting temperature, reaction with *trans*-DDP led to its increase.

In the present work, we have used methodology based on melting temperature measurements to reevaluate the effects on DNA stability of anticancer-active and inactive platinum(II) complexes usually used in the studies of structure–pharmacological activity relationship. In order to better understand the factors affecting the stability of the platinated DNA, we have performed a more systematic study based on the measurements of DNA melting curves at various ionic strengths of the medium in which the platinated natural DNAs or synthetic oligonucleotide duplexes were dissolved.

1. Materials and methods

cis-DDP, *trans*-DDP and [Pt(dien)Cl]Cl were from Lachema a.s. (Brno, Czech Republic). Calf-thymus DNA (42% G + C, molecular mass ca. 2×10^7) was prepared and characterized as described previously [14]. Plasmid pSP73KB (2455 bp) is a derivative of plasmid pSP73 from which the sequence GTACCCGGG has been deleted [15]. It was isolated according to standard procedures and banded twice in CsCl/ethidium bromide equilibrium density gradients. It was linearized by *Eco*RI restriction enzyme (this plasmid contains only one *Eco*RI recognition site). The completeness of the cleavage was verified on 1% agarose minigel. DNAs were reacted with the platinum complexes in 10 mM $NaClO_4$ for 48 h at 37°C in the dark. It was verified by a differential pulse polarographic assay [16] that under these conditions the platinum complexes reacted with DNA quantitatively.

The oligodeoxyribonucleotides 5′-d(CTCCTCC**G**TCTCTTC) [d(CGT)] and 5′-d(GGAAGAGACGGAGGA) [d(ACG)] were purchased from BioVendor (Czech Republic) and purified as described previously [11]. d(CGT) was also modified by [Pt(dien)Cl]Cl so that it contained a single adduct of this platinum complex at the deoxyriboguanosine residue designated in the nucleotide sequence of the d(CGT) in boldface [11]. The nonmodified or platinated d(CGT) was annealed with nonplatinated d(ACG) to form a duplex containing 14 base pairs (bp) and characterized also as described previously [11]. Thus, the duplexes d(CGT)/d(ACG) were prepared so that they remained nonplatinated or contained a single adduct of [Pt(dien)Cl]Cl.

The melting curves of DNAs were recorded by measuring the absorbance at 260 nm using a Beckman DU-8 spectrophotometer. If not stated otherwise, the melting curves were recorded in media containing various concentrations of NaCl and 1 mM Tris-HCl with 0.1 mM EDTA, pH 7.4. The T_{m} value was determined as the temperature corresponding to a maximum on the first-derivation profile of the melting curves. The T_{m} values could be thus determined with an accuracy of ± 0.3°C.

2. Results

In the present study the initial melting experiments were aimed at excluding the possibility that the increase of T_{m} values induced in preparations of vertebrate DNAs by the binding of [Pt(dien)Cl]Cl ([9,13]) was not an artifact caused by their heterogeneity. The preparations of high-molecular-mass DNAs contain a mixture of DNA molecules which differ in their length, base composition and sequence. As a result, the melting curves of such DNA samples may have a relatively broad transition region the midpoint of which need not necessarily characterize melting of any of the molecules contained in the mixture. It could also be difficult to determine precisely relatively small changes in melting temperatures caused by modification by Pt(dien)Cl]Cl.

Therefore, we investigated the melting properties of high-molecular-mass DNA using a homogeneous preparation of identical, linearized molecules of plasmid pSP73KB. This DNA was modified by [Pt(dien)Cl]Cl up to r_b values in the range of 0.01–0.05. This range corresponded approximately to the r_b values at which short, 9–20 bp oligonucleotide duplexes were modified by [Pt(dien)Cl]Cl in the previous experiments [11] showing a reduction of T_{m}. Both nonplatinated and platinated samples of the plasmid DNA in 10 mM $NaClO_4$ with 1 mM Tris-HCl plus 0.1 mM EDTA, pH 7.4 yielded a sharp melting transition so that the T_{m} values could be determined with high precision (Fig. 1A). T_{m} of the nonplatinated DNA was 69.0°C and was increased by 0.7, 1.6 and 3.2°C at $r_b = 0.01$, 0.02 and 0.05, respectively. Thus, we have proved that the observed increase of T_{m} value induced by the modification of high-molecular-mass DNA by [Pt(dien)Cl]Cl was not an artifact caused by the nonhomogeneity of preparations of vertebrate DNAs. For comparative purposes, the melting curves of calf thymus DNA modified by [Pt(dien)Cl]Cl were also measured and the resulting T_{m} values are shown in Fig. 1B. In order to explain the fundamental qualitative difference in the effect of [Pt(dien)Cl]Cl on the melting behavior of short

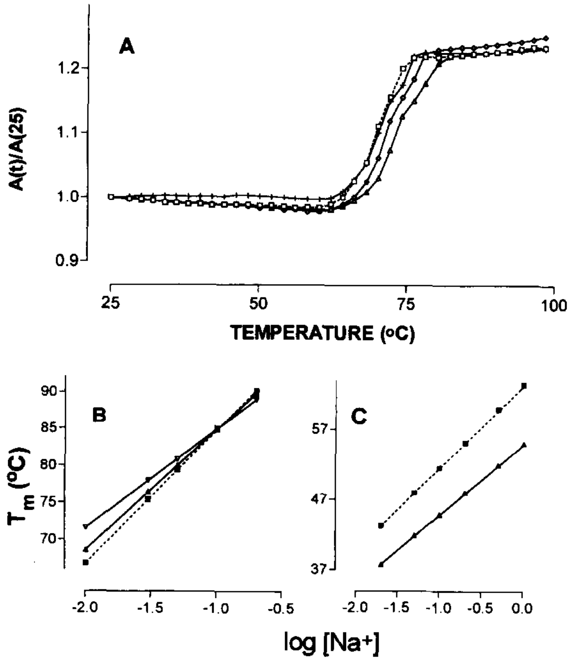

Fig. 1. Melting behavior of DNAs modified by [Pt(dien)Cl]Cl. (A) Melting curves [plots of absorbance at 260 nm measured at elevated temperature, $A(t)$, divided by absorbance at 260 nm measured at 25°C, $A(25)$, vs. temperature] of pSP73KB DNA linearized by *Eco*RI restriction endonuclease measured in 10 mM $NaClO_4$ plus 1 mM Tris-HCl with 0.1 mM EDTA, pH 7.4; r_b values: (-- □ --) 0.0 (control), (– + –) 0.01, (– ◇ –) 0.02, (– △ –) 0.05. B. The dependence of the value of T_m of calf thymus DNA on the concentration of Na^+ measured in media containing various concentrations of NaCl plus 1 mM Tris-HCl with 0.1 mM EDTA, pH 7.4; r_b values: (-- ■ --) 0.0 (control), (– △ –) 0.03, (– ▽ –) 0.1. (C) The dependence of the value of T_m of the synthetic oligodeoxyribonucleotide duplex d(CGT)/d(ACG) (14 bp) containing a single adduct of [Pt(dien)Cl]Cl (see Materials and Methods) on the concentration of Na^+; (-- ■ --), nonplatinated duplex (control), (– △ –) the platinated duplex; other details were the same as in panel (B).

oligonucleotide duplexes (9–20 bp) [11,12] and high-molecular-mass DNAs (Fig. 1A–B and Refs. [9,13]), the melting curves were recorded for samples of calf thymus DNA modified by [Pt(dien)Cl]Cl at $r_b = 0.0$, 0.03 and 0.1. After the platination the samples were dissolved in media containing various concentrations of Na^+ in the range of 0.01–0.2 M at pH 7.4. At low concentration of Na^+ (10 mM), the increase of r_b to the value of 0.1 resulted in an increase of T_m by 6°C (Fig. 1B). If, however, the concentration of Na^+ was increased, the difference between the T_m values of nonmodified and platinated DNAs (ΔT_m) was reduced so that at the Na^+ concentration of 0.1 M, the modification of DNA by [Pt(dien)Cl]Cl had no effect on the T_m value. Interestingly, if the melting curves were measured in the medium containing Na^+ at concentrations higher than 0.1 M, the T_m values of platinated DNA were even lower than those of the control nonmodified DNA (Fig. 1B).

A similar dependence of T_m values on ionic strength was recorded (Fig. 1C) for the oligonucleotide duplex d(CGT)/d(ACG), which was 14 bp in length and contained only one adduct of [Pt(dien)Cl]Cl (the resulting r_b value was 0.033; see Materials and Methods). Already at low concentration of Na^+ (20 mM), T_m value of the modified duplex was lower than that of the control nonmodified duplex by 5°C. In this case, however, further increase of Na^+ concentration resulted in a more pronounced decrease of T_m; at Na^+ concentration of 1.0 M, the T_m value was reduced even by 9°C (Fig. 1C). Semilogarithmic plots of the T_m values vs. Na^+ concentration were linear according to the equations:

$$T_m(\mathrm{noPt}) = 11.91 \log[\mathrm{Na}^+] + 63.51 \qquad (1)$$

$$T_m(\mathrm{dienPt}) = 10.12 \log[\mathrm{Na}^+] + 55.04 \qquad (2)$$

where T_m(noPt) is the melting temperature in °C of control, nonmodified duplex, T_m(dienPt) is the melting temperature in °C of the duplex containing a single adduct of [Pt(dien)Cl]Cl and $[Na^+]$ is the molar concentration of Na^+. The slope of the dependence for the platinated duplex (Eq. (2)) is lower than that of the nonplatinated one (Eq. (1)), which suggests that, hypothetically, at a sufficiently low concentration of Na^+ ($< 1.8 \times 10^{-5}$ M) T_m of this short oligonucleotide containing a single adduct of [Pt(dien)Cl]Cl could be even higher than T_m of the nonmodified duplex.

Thus, it is evident from the melting experiments performed with DNAs modified by [Pt(dien)Cl]Cl that the qualitative character of the effect of the modification of DNA by [Pt(dien)Cl]Cl on T_m (i.e. whether T_m is increased or reduced due to the DNA platination) depends on (i) the concentration of cations in the medium in which the sample of the modified DNA was dissolved and melted and (ii) the length of the double helix.

Further experiments were performed with high-molecular-mass DNA isolated from calf thymus modified globally by bifunctional *cis*-DDP and its *trans* isomer. The effect of *trans*-DDP on T_m measured at different concentrations of Na^+ (Fig. 2A) was qualitatively similar to that of [Pt(dien)Cl]Cl (cf. Fig. 1B and 2A). At a low concentration of Na^+ (0.01 M), the increase of r_b also resulted in an increase of T_m; this increase was, however, more pronounced than in the case of DNA modified by [Pt(dien)Cl]Cl (at $r_b = 0.1$ by 9°C as compared with only 6°C for DNA modified by [Pt(dien)Cl]Cl). With increasing Na^+ concentration above 0.01 M ΔT_m decreased. At Na^+ concentration of ca. 0.1 M the modification of DNA by *trans*-DDP had no effect on T_m and at Na^+ concentrations higher than 0.1 M T_m was lower than that of the nonmodified DNA.

Melting properties of calf thymus DNA modified by *cis*-DDP were different (Fig. 2B). Already in the medium of low ionic strength (0.01 M Na^+) the binding of *cis*-DDP resulted in a decrease of T_m and ΔT_m was further enhanced with increasing concentration of Na^+. For calf thymus DNA, nonplatinated or modified by *cis*-DDP, semilogarithmic plots of T_m vs. Na^+ concentration were again linear and could be described by the following equations:

$$T_m(\text{noPt}) = 17.98\log[Na^+] + 102.79 \qquad (3)$$

$$T_m(\text{cisPt}, r_b = 0.1) = 13.46\log[Na^+] + 90.21 \qquad (4)$$

where $T_m(\text{cisPt}, r_b = 0.1)$ is T_m in °C of DNA modified by *cis*-DDP at $r_b = 0.1$. Fig. 2B and Eqs. (3) and (4) demonstrate that the slope of these dependencies decreases with increasing level of the modification. It can be suggested on the basis of the extrapolation using the Eqs. (3) and (4) that the effect of *cis*-DDP on T_m values could be reversed at Na^+ concentrations lower than about 5 mM, i.e. that under these conditions the modification by *cis*-DDP could even stabilize DNA. However, it is difficult to verify this assumption experimentally, since the control of ionic strength at values comparable with DNA concentration is rather problematic. We recorded melting curves of samples of calf thymus DNA modified by *cis*-DDP at $r_b = 0.0$, 0.03 and 0.1, which were, before the melting experiment, transferred into the medium of 4 mM NaCl with 1×10^{-4} M EDTA, pH 7, using an exhaustive dialysis. The melting transitions of the platinated DNAs were much broader than that of nonmodified DNA and thus the small increase of T_m (by 2°C), which was observed in the platinated samples relative to T_m of nonmodified DNA, represents only a qualitative confirmation of the above assumption.

3. Discussion

It has been demonstrated that formation of monofunctional platinum(II) adducts induces distortions in DNA duplexes [9,11,13,17] and changes their thermal stability [9,11–13]. In general, the monofunctional binding of [Pt(dien)Cl]Cl can affect the stabil-

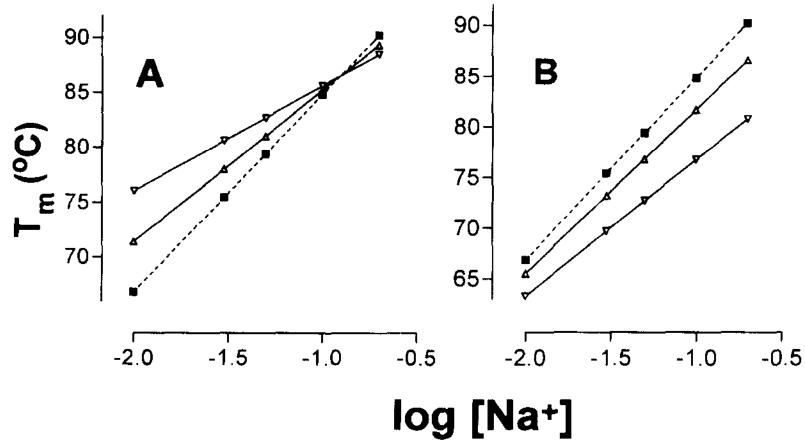

Fig. 2. Plots of T_m values of calf thymus DNA modified by *trans*-DDP (A) or *cis*-DDP (B) on the concentration of Na^+ measured in media containing various concentrations of NaCl plus 1 mM Tris-HCl with 0.1 mM EDTA, pH 7.4; r_b values: (-- ■ --) 0.0 (control), (– △ –) 0.03, (– ▽ –) 0.1.

ity of the DNA duplex in two ways: attachment of positive charges of the platinum(II) moiety constitutes a stabilizing effect, whereas conformational distortions destabilize the double helix. The observed net decrease of T_m induced in short duplexes by the presence of a single monofunctional adduct (Fig. 1C and Refs. [11,12]) gives evidence that in these double-helical oligonucleotides the destabilizing effect dominates any stabilizing factors. On the other hand, under similar ionic conditions the modification of high-molecular-mass DNA by monofunctional [Pt(dien)Cl]Cl results in an increase of T_m. Only at a relatively high ionic strength of the medium (0.2 M Na^+) a decrease of T_m was observed (Fig. 1B). Thus, the results presented in Fig. 1B indicate that an enhancement of the concentration of Na^+ weakens the stabilizing effects of [Pt(dien)Cl]Cl binding.

It has been shown [18] that cations present in the medium stabilize DNA against thermal denaturation by decreasing electrostatic repulsion of negative charges of phosphate groups located at the complementary strands. Introduction of a positive charge into the DNA molecule, e.g. by binding of a charged ligand, results in an additional stabilization of the DNA duplex. This stabilization effect depends on the concentration of cations in the medium. It is stronger at low ionic strength and practically disappears at sufficiently high concentrations of the cations.

The stabilizing contribution, which is due to the binding of a positively charged ligand to DNA, can be expressed by Eq. (19)

$$\Delta T_m = T_m - T_{m,0} = 2 r_b \frac{\lambda \psi_1 e}{\Delta S_0} \qquad (5)$$

Here T_m and $T_{m,0}$ are melting temperatures of modified DNA at given r_b and pure DNA (at $r_b = 0$), respectively, determined at the same ionic strength. ψ_1 is the value of electrostatic potential induced by the negative charges located on one DNA strand in position of positive charges e of the bound ligand. This function can be approximated [19] by the values of electrostatic potential in the positions of phosphate groups of the other strand, ψ [18]. A value of 0.22 for the reducing factor λ [20] was taken in accordance with previous calculations [18,19,21]. For the change of entropy corresponding to the helix–coil transition, ΔS_0, a value of 22 entropy units was used [19,21] (its value does not practically depend on the DNA base composition [22]).

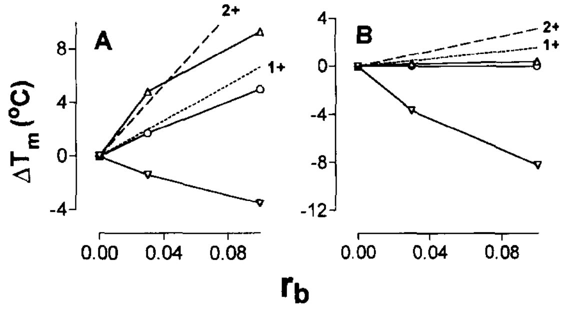

Fig. 3. Comparison of the electrostatic stabilization effect of ligands bearing 1+ and 2+ charges calculated using Eq. (5) (dashed lines designated as 1+ and 2+, respectively) with experimental values of ΔT_m of calf thymus DNA modified by [Pt(dien)Cl]Cl (– ○ –), *trans*-DDP (– △ –) or *cis*-DDP (– ▽ –). The concentration of Na^+ was 0.01 M (A) and 0.2 M (B). In the melting experiments the concentration of Na^+ was adjusted by addition of NaCl to DNA samples incubated with platinum compounds in 10 mM sodium perchlorate.

Fig. 3 shows changes in the T_m values (ΔT_m) determined for the three complexes investigated, *cis*-DDP, *trans*-DDP and [Pt(dien)Cl]Cl at two extreme Na^+ concentrations used in our experiments, 0.01 and 0.2 M. The experimental data are compared with ΔT_m calculated using Eq. (5), which would be induced by introduction of 1 + or 2 + charges born by the coordinated platinum complexes, not considering other stabilization or destabilization effects of the ligands (dashed lines in Fig. 3).

Importantly, the bifunctinal coordination of both diamminedichloroplatinum(II) isomers introduces always two positive charges per platination event. On the other hand, for monofunctional attachment of any of the three platinum(II) compounds the value of mean charge introduced per platinated base residue can vary between 1 + and 2 + depending on the extent of hydrolysis of the noncoordinated leaving group and on the ionization state of this group if it was hydrolyzed. In the case of [Pt(dien)Cl]Cl this concerns the second chloride coordinated in the outer sphere. The monofunctional binding of *cis*-DDP and its *trans* isomer should also be considered since both isomers form a significant amount of monofunctional adducts even after long incubation of the platinum complex with DNA (48 h at 37°C in a chloride-free medium) [8]. Thus, the sector between the dashed lines designated as 1 + and 2 + in Fig. 3 defines the range of ΔT_m values which could be expected if electrostatic contributions of charges of the ligands only affected the melting behavior of platinated DNA.

The ΔT_m values determined experimentally for DNA modified by *cis*-DDP and [Pt(dien)Cl]Cl lied below this sector at both concentration of Na^+ (0.01 and 0.2 M) (Fig. 3A, B). DNA modified by *trans*-DDP yielded qualitatively similar results only in the medium of 0.2 M Na^+ (Fig. 3B). In 0.01 M Na^+ (Fig. 3A) the experimentally determined value of ΔT_m at $r_b = 0.03$ was, however, slightly higher than the value calculated for the charge 2 + introduced per platination event. The lowering of the experimental values of ΔT_m was radically greater if DNA was modified with *cis*-DDP in comparison with the effects of modification by either of the two other complexes (Fig. 3).

The melting behavior of DNA modified by the platinum complexes observed in this work is consistent with at least two principal contributions from the platinum adducts to the global stability of DNA: (i) the stabilizing effect of positive charges located at platinum atoms and (ii) 'specific' (non-electrostatic) effects, which may be stabilizing or destabilizing depending on the nature of the adduct formed (this includes conformational distortions, formation of cross-links, etc.) [21]. Thus, the results shown in Fig. 3 support the view that the nonelectrostatic effect of *cis*-DDP and [Pt(dien)Cl]Cl involve destabilization of DNA at both low and high concentration of Na^+. The global destabilization of DNA by nonelectrostatic effects is also apparent for *trans*-DDP adducts, if the melting experiments were carried out at higher Na^+ concentrations. Changes in T_m induced by *trans*-DDP at the lower Na^+ concentration cannot be interpreted unequivocally, nevertheless, the results do not exclude the above conclusion about a destabilizing nonelectrostatic effect of *trans*-DDP.

Thus, it can be suggested that in the case of high-molecular-mass DNA modified by [Pt(dien)Cl]Cl and present in the medium of a low Na^+ concentration the electrostatic stabilizing effect dominates and therefore an increase of T_m is observed in a broad range of r_b values (Fig. 1B). At higher concentrations of Na^+ (> 0.1 M) the negative charges of DNA phosphate groups are efficiently neutralized by the cations present in the medium so that an additional contribution of the positive charge from the platinum moiety of the [Pt(dien)Cl]Cl adduct to the stability of high-molecular-mass DNA is much less expressed. Due mainly to end effects, short oligonucleotide duplexes are markedly less stable than high-molecular-mass DNAs so that the same destabilization factor (presumably a conformational distortion [9,11,13,17]) introduced by the monofunctional platinum binding has more radical consequences on the overall stability of the less stable duplex. This suggestion is supported by the results of our previous paper [11]. Melting temperatures of longer duplexes (20 bp in length) are less reduced due to the formation of the same number of [Pt(dien)Cl]Cl adducts per one base pair than those of shorter oligonucleotide duplexes.

In the case of DNA modification by bifunctional *cis*-DDP and its *trans* isomer, formation of interstrand cross-links [23,24] may be another factor contributing to the stability of the duplex. The covalent interstrand cross-links of *cis*- or *trans*-DDP do not allow complete separation of the two strands of DNA above the melting temperature. The effect of *trans*-DDP on the thermal stability of DNA measured at various concentrations of Na^+ was qualitatively similar to that of [Pt(dien)Cl]Cl (cf. Fig. 1B and Fig. 2A). However, DNA is stabilized more efficiently by *trans*-DDP than by [Pt(dien)Cl]Cl at low concentrations of Na^+. The higher stabilizing effect of *trans*-DDP need not be due only to a higher number of positive charges located at the bound platinum atom. Another contribution to the higher stabilizing efficacy of *trans*-DDP can come from its ability to form interstrand cross-links in DNA [23,24]. The latter explanation is strongly supported by the fact that monofunctional [Pt(dien)Cl]Cl cannot form in DNA interstrand cross-links. A higher stability of DNA modified by *trans*-DDP in comparison with DNA modified by [Pt(dien)Cl]Cl observed at 0.01 M concentration of Na^+ could be also interpreted to mean that distortions induced by *trans*-DDP destabilize DNA less efficiently than those induced by [Pt(dien)Cl]Cl, but this hypothesis has no experimental support.

Antitumor *cis*-DDP decreases T_m of high-molecular-mass DNA already at low ionic strength (0.01 M Na^+), which suggests that distortions induced by this drug destabilize DNA more efficiently than those induced by both clinically ineffective compounds, [Pt(dien)Cl]Cl or *trans*-DDP. Nevertheless, the modification by this complex can increase the T_m value of DNA at a very low ionic strength (ca. 5×10^{-4} M Na^+). In the absence of effective screening of the negative charges of DNA phosphate groups, the stabilizing effects of the positive charge of the platinum moiety of *cis*-DDP could be no longer obscured by the stabilizing effect of high concentration of cations present in the medium. In other words, only at a very low concentration of Na^+ (ca. 5×10^{-4} M) the stabilizing effects of the 2 + charge of the platinum moiety of *cis*-DDP along with DNA interstrand cross-linking could overcompensate the destabilizing effects of conformational distortions induced in DNA by this drug.

The present paper shows that the melting of DNA modified by platinum complexes strongly depends on the concentration of the cations in the medium which neutralize the negative charges of the phosphate groups of DNA. The results suggest that at least three factors affect the stability of DNA upon the binding of platinum complexes: stabilization effects of the positive charge on the platinum moiety and of interstrand cross-links, and destabilization effect of conformational distortions in DNA. This work also confirms that the measurement of melting curves of DNA modified by low-molecular-mass compounds can provide a useful information on the mode how these compounds change some physical properties of this biopolymer. However, in order to compare and interpret the melting behavior of DNA modified by different compounds, a great attention has to be also paid to the composition of the medium in which the melting experiments are carried out.

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