Reactivity of transplatin-modified oligonucleotides in triple-helical DNA complexes

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The purpose of this work was to gain insight into the formation of interstrand cross-links in DNA triple helices resulting from the association between double-stranded DNAs and the complementary oligonucleotides containing a single transplatin monofunctional adduct either *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ or *trans*-$[Pt(NH_3)_2(dC)Cl]^+$. Depending upon its location along the oligonucleotide, a platinated guanine residue increases or decreases the thermal stability of the platinated triplexes, as shown on model systems in which the transplatin monofunctional adduct was replaced by a diethylenetriamineplatinum(II) adduct. The interstrand cross-linking reaction has been studied in triplexes containing a single transplatin monofunctional adduct as a function of several parameters. The rate of closure of the monofunctional adduct into an interstrand cross-link depends upon the nature of the adduct but not strongly on its location along the Hoogsteen strand. The closure of *trans*-$[Pt(NH_3)_2(dC)Cl]^+$ is faster than that of *trans*-$[Pt(NH_3)_2(dG)Cl]^+$. Whereas the closure of *trans*-$[Pt(NH_3)_2(dC)Cl]^+$is hardly affected by the presence of a high concentration of NaCl in the medium, the closure of *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ is largely slowed down. These results are discussed in the context of the potential use of the platinated oligonucleotides to modulate gene expression.

Réactivité d'oligonucléotides modifiés par le transplatine dans des triples hélices d'ADN. L'objectif de ce travail est d'élucider certains aspects de la réaction de formation d'adduits interbrins dans des triples hélices d'ADN formées par l'association entre des doubles hélices d'ADN et les oligonucléotides complémentaires contenant un adduit monofonctionnel du transplatine, soit *trans*-$[Pt(NH_3)_2(dG)Cl]^+$, soit *trans*-$[Pt(NH_3)_2(dC)Cl]^+$. Selon sa position dans l'oligonucléotide, une guanine platinée augmente ou diminue la stabilité thermique des triples hélices platinées. Ceci a été montré à l'aide de modèles dans lesquels l'adduit monofonctionnel du transplatine est remplacé par un adduit du diéthylènetriamineplatine(II). La réaction de formation d'adduits interbrins a été étudiée dans des triplexes platinés en fonction de plusieurs paramètres. La vitesse de formation de l'adduit interbrin à partir de l'adduit monofonctionnel dépend de la nature de l'adduit mais peu de sa position dans le brin Hoogsteen. La réaction du *trans*-$[Pt(NH_3)_2(dC)Cl]^+$ est plus rapide que celle du *trans*-$[Pt(NH_3)_2(dG)Cl]^+$. La présence d'une grande concentration de NaCl dans le milieu réactionnel n'a presque aucun effet sur la réaction du *trans*-$[Pt(NH_3)_2(dC)Cl]^+$, mais ralentit fortement la réaction du *trans*-$[Pt(NH_3)_2(dG)Cl]^+$. Ces résultats sont discutés dans le cadre de l'utilisation potentielle des oligonucléotides platinés pour moduler l'expression génique.

Numerous studies have been recently devoted to oligonucleotides able to recognize specifically sequences within double-stranded DNA *via* the formation of triple helices, one of the aims being to use the oligonucleotides for modulation of gene expression, the so-called antigene strategy.[1–3] A major difficulty in this strategy is that the triple helices have a rather low thermodynamic stability and consequently the cellular machinery can displace the bound oligonucleotides. Several ways have been proposed to increase the binding strength of the oligonucleotides. Attachment of a reactive group to the oligonucleotides that could induce an efficient irreversible reaction with the target DNA seems promising.[4,5] Among the reagents able to react spontaneously, platinum(II) derivatives have been used with success.[6] Recently, we have shown that oligonucleotides containing a single monofunctional transplatin [*trans*-diamminedichloroplatinum(II)] adduct can bind to complementary duplexes and that the monofunctional adducts further react and form interstrand cross-links.[7]

Our previous results were obtained with a homopyrimidine sequence containing a single monofunctional *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ or *trans*-$[Pt(NH_3)_2(dC)Cl]^+$ adduct.[7] The purpose of the present work was to analyse in more detail the interstrand cross-linking reaction. We have extended our first results to another sequence containing several purine residues and we try to answer the following questions. Can the presence of a monofunctional adduct increase the thermal stability of the triplex? The monofunctional adduct brings a positive charge, which could decrease the repulsions of the negatively charged phosphodiester backbones. Does the reactivity of the monofunctional adduct depend on its location along the oligonucleotide? What is the nature of the reacting entity during the closure of the monofunctional adduct into an interstrand cross-link?

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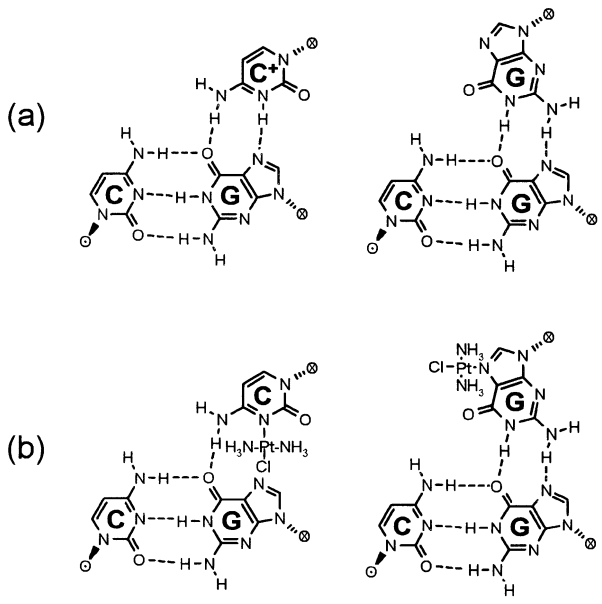
Results and Discussion

Thermal stability of the platinated triplexes

In order to study the effect of the monofunctional adducts on the thermal stability of the triplexes, oligonucleotides **1** and **2** (Fig. 1) were reacted with dien-Pt [iododiethylenetriamine-platinum(II) iodide]. Dien-Pt forms stable monofunctional adducts, $[Pt(dien)(dG)]^{2+}$. Several results have shown that they mimic to some extent the transplatin monofunctional adducts.[8,9] In the triplexes **A.1** and **A.2** resulting from the binding of oligonucleotides **1** or **2** with duplex **A**, respectively, the Hoogsteen strands stand in the major groove of the double helix **A** in a parallel orientation with respect to the homopurine strand[10] and the N7 of the G residues on the outside of the triplexes are not involved in hydrogen bonds (Fig. 1). Platination at the N7 position of guanine residues does not interfere directly with the hydrogen bonds. Moreover, replacement of G residues by 7-deazaguanine residues presents the advantages of reducing the number of sites that are highly reactive with platinum(II) complexes without altering the base pairing and also of preventing self-association of oligonucleotides **1** or **2**. The experiments were not done with the triplexes **B.3** and **B.4**. The platination of a C residue at N3 has for consequence the loss of a hydrogen bond (Fig. 1) and thus is a destabilizing factor for the triplex.



10 15
1: 5'-TTTT^{m}CTTTTGgggggT-3'

2: 5'-TTTT^{m}CTTTTgggggGT-3'

A
5'-CCACTTTTTAAAAGAAAAGGGGGGACTGG-3'
3'-GGTGAAAAATTTTCTTTTCCCCCCTGACC-5'

3: 5'-GTTTTTCTTTCTTTTCTTT-3'

4: 5'-TTTTTCTTTCTTTTCTTT-3'

B
5'-GTCAGAAAAAGAAAGAAAAGAAACG-3'
3'-CAGTCTTTTTCTTTCTTTTCTTTGC-5'

Fig. 1 Top: Base triplets formed between a Watson–Crick base pair C≡G and (*a*) a third base G or C (Hoogsteen pairing) or (*b*) a transplatinum-modified third base G or C. Bottom: Sequences of the oligonucleotides used in this study and their abbreviations. The symbol g stands for 7-deazaguanine and ^{m}C for 5-methylcytosine. Double-underlined symbols indicate bases that were platinated. Underlined bases indicate the zone of the duplexes **A** and **B** complementary to the oligonucleotides **1**, **2** and **3**, **4**, respectively

Oligonucleotide **2** contains one G residue. In the platination reaction at acidic pH only this residue was reactive with dien-Pt (results not shown). Oligonucleotide **1** contains two G residues. In our experimental conditions the G residue located at position 10 was much more reactive than the G residue at position 15, as clearly shown by the reaction of the platinated oligonucleotide **1**^{d} with DMS (dimethyl sulfate, Fig. 2). The platination of the guanine residues prevents the reaction of DMS at the N7 of these residues and consequently no strand cleavage occurs during the piperidine treatment.[11] Although a structural effect cannot be completely excluded, the preferential platination of the G10 residue is probably due to an electrostatic effect along the oligonucleotide.[12–15]

The thermal stabilities of the triplexes **A.1**, **A.2** and the corresponding triplexes **A.1**^{d} and **A.2**^{d} containing a single monofunctional $[Pt(dien)Cl(dG)]^{2+}$ adduct have been studied by measuring the absorbances of the triplexes at 260 nm. It is known that the triplex resulting from the association of the duplex **A** and the oligonucleotide **1** in which all the deazaguanine residues have been replaced by guanine residues present a biphasic melting with first dissociation of the Hoogsteen strand and then dissociation of the duplex **A**.[10] In Fig. 3 are only shown some data relative to the dissociation of the Hoogsteen strands. The two triplexes **A.1** and **A.2** melt cooperatively and have the same T_m (30 °C). The triplex **A.2**^{d} melts also cooperatively and the T_m is slightly increased (35 °C). The melting of triplex **A.1**^{d} (the platinated G residue at position 10) occurs at lower temperature and is less cooperative, which reveals a strong destabilizing effect of this platinated residue. The conclusion of this experiment is that a platinated G residue within the Hoogsteen strand has two

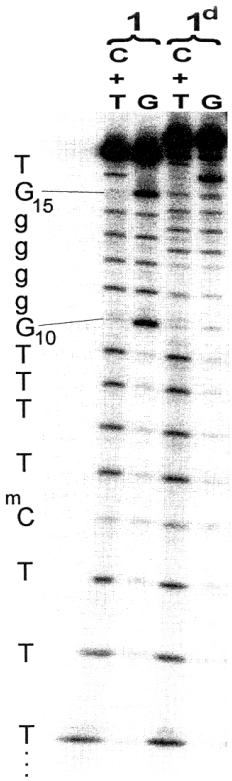


Fig. 2 Identification of the platinated products resulting from the reaction of the oligonucleotide **1** with dien-Pt. Lanes (C + T) and (G) refer to the Maxam–Gilbert sequencing reactions with hydrazine and dimethyl sulfate, respectively. Lanes 1 and 1^{d} refer to oligonucleotide **1** containing 0 or 1 platinum adduct, respectively

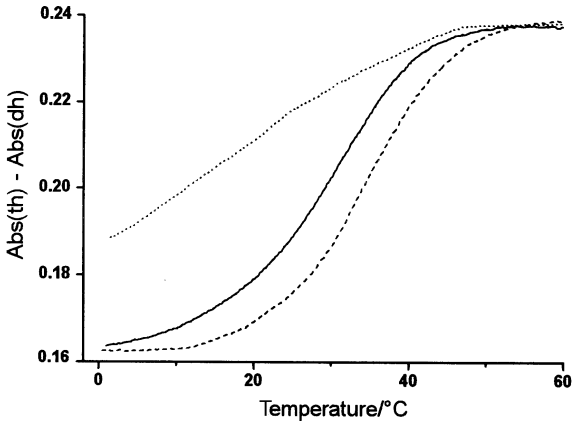


Fig. 3 Melting curves of the triplexes **A.1** and **A.2** containing or not a single $[Pt(dien)(dG)]^{2+}$ adduct, in 50 mM $NaClO_4$, 10 mM $Mg(ClO_4)_2$, 10 mM phosphate buffer pH 6.8. For the sake of clarity, the absorbance of the duplex was subtracted from the absorbance of the triplexes. (——) Triplex **A.1**; (····) triplex **A.1**^{d} with a single adduct at G10; (– – –) triplex **A.2**^{d} with a single adduct at G15. The melting curve of triplex **A.2** was identical to that of triplex **A.1** and thus is not plotted

opposite effects. On the one hand it stabilizes the triplex, probably by its positive charges, and on the other hand it destabilizes the triplex by a steric effect. A similar effect is expected from the monofunctional *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ adduct.

Finally, we have also studied the thermal stability (not shown) of the triplex formed by the association of the duplex **A** with the oligonucleotide **1** containing two adducts (at positions 10 and 15). It was identical to that of triplex **A.1**^{d}, thus confirming the major destabilizing effect of the adduct at position 10.

Closure of the monofunctional adduct into an interstrand cross-link within the triplexes

The next step was to determine the rate of the cross-linking reaction into triplexes in which the Hoogsteen strands contained a single monofunctional *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ adduct. As in the case of dien-Pt, transplatin reacted first with the G10 residue and then with the G15 residue within oligonucleotide **1** (results not shown). The platinated Hoogsteen strands, ^{32}P-labelled at the 5′ end, were mixed with the complementary duplexes. The triplexes **A.1**^{t} and **A.2**^{t} were incubated at 25 °C. At various times aliquots were withdrawn and analysed by gel electrophoresis under denaturing conditions. It has been previously shown that oligonucleotides containing intrastrand or interstrand cross-links are easily separated by this technique.[16,17] As a function of time, the intensity of the initial band (Hoogsteen strand) decreases as new bands, which migrate more slowly, appear (Fig. 4, right). This indicates the formation of interstrand cross-links. In the case of triplex **A.2**^{t} (the monofunctional adduct is located one residue away from the 3′ end), there is mainly one slowly migrating band. The time ($t_{1/2}$) at which one-half of the monofunctional adduct has disappeared is about equal to 20 h. It has been verified that the competitive suicide reaction, that is dissociation of the Hoogsteen strand followed by the formation of intrastrand cross-links within the free oligonucleotide, was a minor event [treatment of the incubated sample (24 h) for 10 min in 10 mM thiourea removed almost completely the monofunctional adduct (lane T), which excludes the formation of intrastrand cross-links].

The closure of the monofunctional adduct into an interstrand cross-link within the triplex **A.1**^{t} is apparently slower (Fig. 4, left). However, at 25 °C, the triplex is probably partially dissociated. This is deduced from the results on the

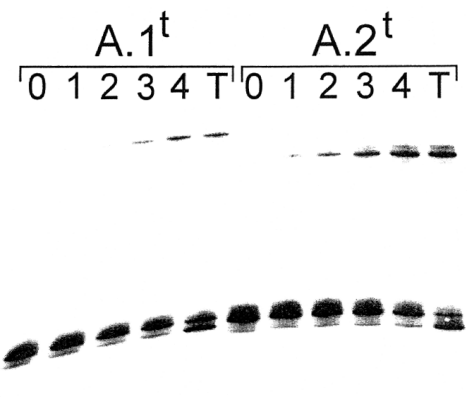


Fig. 4 Kinetics of closure of the monofunctional *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ adducts within the two triplexes: **A.1**^{t} (adduct at G10) and **A.2**^{t} (adduct at G15). Autoradiogram of a denaturing 24% polyacrylamide gel. Lanes 0–4 refer to incubation times of 0, 3, 6, 12 and 24 h, respectively, at 25 °C and in 10 mM $Mg(ClO_4)_2$, 10 mM phosphate buffer pH 6.8. Lanes T refer to treatment of the products with 10 mM thiourea for 10 min at 37 °C after 24 h of incubation

thermal stability of the triplex containing a single dien-Pt (Fig. 3) and further confirmed by the thiourea treament of the triplex after it had been incubated 24 h. The formation of intrastrand cross-links (suicide reaction) was not negligible, as revealed by gel electrophoresis (Fig. 4, left, lane T). Taking into account the amount of intrastrand cross-links, $t_{1/2}$ of the monofunctional adduct within the triplex is about 24 h.

In previous work,[7] we did similar experiments with the triplexes **B.3**^{t} and **B.4**^{t} in which the Hoogsteeen strands contained the single monofunctional adducts *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ or *trans*-$[Pt(NH_3)_2(dC)Cl]^+$, respectively. These experiments, performed at 37 °C, were now repeated at 25 °C (see also Fig. 5). In these conditions there were no suicide reactions and the values of $t_{1/2}$ of the monofunctional adducts are 15 h and 9 h, respectively.

These results call for some comment. Concerning the platinated G residues in the triplexes, the rate of closure of this monofunctional adduct into an interstrand cross-link is not strongly affected by the location of the adduct along the Hoogsteen strand (the $t_{1/2}$ are respectively equal to 15, 20 and 24 h for the adduct located at the 5′ end, at one residue before the 3′ end and in the middle of the sequence). The cross-linking reaction implies a rotation of the platinated G residue about the glycosidic bond to have the platinum residue near the attacking G residue within the homopurine strand. Our results suggest that this rotation is not dramatically more hindered in the middle of the triplex than at its ends. One explanation for these results is that the triplex is locally distorted at the level of the monofunctional adduct. In favour of a local distortion is the fact that a monofunctional dien-Pt in the middle of the sequence induces a large decrease of the thermal stability of the triplex. The consequence of this distortion is an increase of freedom of the platinated G residue, which makes its conversion from the *anti* to the *syn* conformation easier.

The presence of a platinated C residue decreases also the thermal stability of the triplex. This is due to the loss of the pairing between the monofunctional adduct and the complementary G residue and, possibly, to a local distortion due to a steric hindrance. On the other hand, the monofunctional adduct is located near the attacking G residue within the homopurine strand which might explain to some extent that the closure of the monofunctional C adduct is faster than that of the monofunctional G residue.

Interstrand cross-linking reaction within the triplexes in the presence of NaCl

Another important point was to determine the nature of the reactive entity. Most reactions between DNA and cisplatin or

transplatin proceed through solvent-associated intermediates.[18] We wondered whether the location of the monofunctional adducts, and particularly *trans*-$[Pt(NH_3)_2(dC)Cl]^+$, in the triplexes favoured a direct displacement of chloride by the attacking base residue rather than the intermediacy of the aquated species. To choose between the two mechanisms the rate of the interstrand cross-linking reaction was determined in the presence of NaCl instead of $NaClO_4$. The experiments were only done with the two triplexes **B.3**^{t} and **B.4**^{t} in NaCl or $NaClO_4$ and in the salt range 0.15–0.5 M. It has been verified that no changes in the conformation of these two triplexes as a function of the concentration and nature of salt were detected by circular dichroism, whereas changes were detected for the two triplexes **A.1**^{t} and **A.2**^{t} (not shown).

In the triplex **B.3**^{t}, *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ is located at the 5′ end of the Hoogsteen strand and thus largely exposed to the solvent. The rate of the interstrand cross-linking reaction was the same in 0.15 and 0.5 M $NaClO_4$ but decreased by about a factor of 5 in 0.5 M NaCl (Fig. 5, left). This suggests that hydrolysis of chloride occurs for closure of the monofunctional *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ adduct. In the triplex **B.4**^{t}, *trans*-$[Pt(NH_3)_2(dC)Cl]^+$ is located in the middle of the Hoogsteen strand. The rate of the interstrand cross-linking reaction was unchanged in the two $NaClO_4$ solutions and slightly decreased in 0.5 M NaCl (Fig. 5, right). Two explanations can be proposed. Although not paired with the complementary G residue, the monofunctional *trans*-$[Pt(NH_3)_2(dC)Cl]^+$ adduct is inserted into the triplex and thus is not in contact with chloride ions. The limiting step for the closure of the adduct into an interstrand cross-link is the formation of the aquated entity. The second explanation is a direct displacement of chloride by the attacking base residue. We favour the first explanation but we cannot exclude the second one.

Conclusions

From the results here presented and those previously reported[7] some conclusions can be drawn on the interstrand cross-linking reaction in triplexes containing a monofunctional *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ or *trans*-$[Pt(NH_3)_2(dC)Cl]^+$ adduct. These adducts do not modify the orientation of the third strand in the triplexes. The reaction is specific in the sense that we did not observe any binding of the platinated single-stranded oligonucleotides to a double-stranded DNA that did not contain the target sequence[7]. The rate of the interstrand cross-linking reaction is faster with the *trans*-$[Pt(NH_3)_2(dC)Cl]^+$ adduct than with the *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ adduct. Another advantage of the *trans*-$[Pt(NH_3)_2(dC)Cl]^+$ adduct is that the rate of the reaction is almost unchanged by addition of NaCl. This suggests that in the triplex the adduct is hardly accesible to small molecules and thus is not expected to be attacked by compounds such as gluthatione or biomolecules that react strongly with platinum(II) complexes. Several improvements have to be achieved in order to use these platinated oligonucleotides as tools in molecular biology or in the context of the antigene strategy. It is still difficult to platinate a given C residue within an oligonucleotide containing several C residues or several C and G residues. It seems reasonable to think that this difficulty will be solved by the automated solid-phase synthesis of site-specific-platinated oligonucleotides,[19,20] in complement to other approaches.[21] Another point is related to the destabilization of the triplexes due to the monofunctional adducts. It is known that triplexes are stabilized by replacement of the oligodeoxyribonucleotides (Hoogsteen strands) by oligo(2′-*O*-methylribonucleotides).[22,23] Preliminary results (not shown) indicate that this replacement does not prevent the interstrand cross-linking reaction from occurring. Finally, for *in vivo* applications, the rate of the interstrand cross-linking reaction should be increased. This cannot be done as long as the reaction proceeds through solvent-associated intermediates. Work is in progress to find conditions that could favour a direct displacement of the leaving groups of the platinum residue by the attacking base residue.

Experimental

General

The oligodeoxyribonucleotides purchased from Eurogentec were purified as previously described.[24] Their sequences are as shown in Fig. 1. The triplexes obtained by mixing equimolar amounts of duplex **A** with the oligonucleotides **1** or **2** or duplex **B** with oligonucleotides **3** or **4** were respectively named **A.1**, **A.2**, **B.3** and **B.4**. Transplatin was from Johnson Matthey (U.K.), and iododiethylenetriamineplatinum(II) iodide (dien-Pt) was kindly provided by Pr. Reedijk (Leiden, The Netherlands). T4 polynucleotide kinase was purchased from New England Biolabs. The radioactive products were from Amersham. Other chemicals were from Sigma except acrylamide, which was from Appligene.

Platination of the oligonucleotides

The platination reactions were done as previously described with minor modifications.[25] To obtain oligonucleotides **1**^{t}, **2**^{t} and **3**^{t} containing a single *trans*-$[Pt(NH_3)_2(dG)Cl]^+$, oligonucleotides **1**, **2** and **3** (35 μM) were incubated with *trans*-$[Pt(NH_3)_2(H_2O)Cl]^+$ (generated by allowing transplatinum to

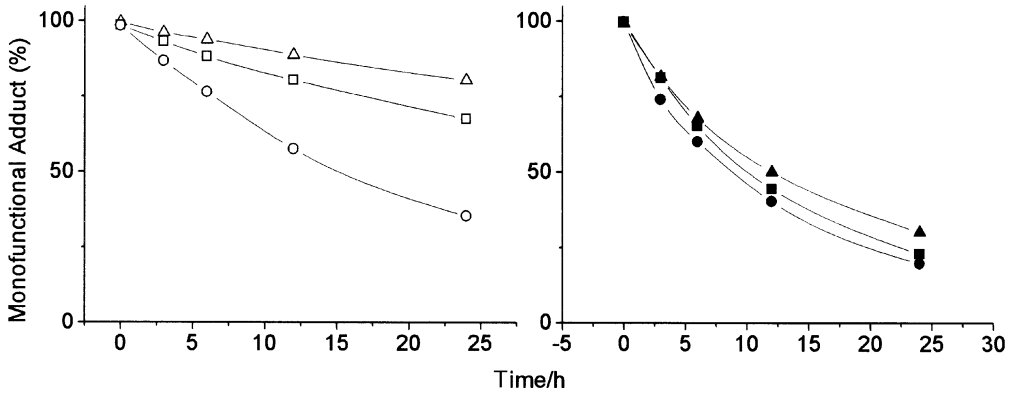


Fig. 5 Kinetics of closure of monofunctional *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ (left) and *trans*-$[Pt(NH_3)_2(dC)Cl]^+$ (right) adduct within the triplexes **B.3**^{t} and **B.4**^{t}, respectively, at 25 °C and in 5 mM $MgClO_4$, 5 mM acetate buffer pH 5.0, and $NaClO_4$ or NaCl as indicated below. The percentages of monofunctional adduct were calculated from the ratios of the sums of the intensities of the slowly migrating bands over the sum of the intensities of all the bands on the corresponding autoradiograms. (△/▲) 0.5 M NaCl; (□/■) 0.15 M NaCl; (○/●) 0.5 and 0.15 M $NaClO_4$

react with $AgNO_3$) at 37 °C in 10 mM $NaClO_4$, 10 mM acetate buffer pH 3.6. Oligonucleotide **1** was incubated at a platinum-to-oligonucleotide molar ratio $r_i = 1.5$ for 60 min, oligonucleotides **2** and **3** at $r_i = 4$ for 30 min. The oligonucleotide $\mathbf{4}^t$ containing the monofunctional adduct trans-$[Pt(NH_3)_2(dC)Cl]^+$ was obtained by reacting oligonucleotide **4** (42 μM) with *trans*-$[Pt(NH_3)_2(H_2O)Cl]^+$ at $r_i = 4$ for 30 min at 37 °C in 10 mM $NaClO_4$. The platinated oligonucleotides were purified by strong anion exchange column (MonoQ HR 5/5, Pharmacia) in a 10 mM NaOH, 0.3–1 M NaCl gradient over 40 min. The purified oligonucleotides were dialysed for 2 h, at 4 °C, against 3 × 500 ml of 0.1 M NaCl, 5 mM phosphate buffer at pH 7.5 and stored at −20 °C.

Oligonucleotides $\mathbf{1}^d$ and $\mathbf{2}^d$ containing a single $[Pt(dien)(dG)]^{2+}$ monofunctional adduct were obtained by reacting oligonucleotides **1** and **2** (35 μM) with $[Pt(dien)(H_2O)]^{2+}$ at $r_i = 3$ or 1.2, respectively, for 10 min at 37 °C in 10 mM $NaClO_4$, 10 mM acetate buffer pH 3.6. The platinated oligonucleotides were purified as above and the fractions were neutralized with acetic acid and Tris-HCl and further desalted on Sep-Pak C18 cartridges (Waters). Location of the adducts was verified by the non-reactivity of DMS with the platinated guanine residues.

Melting curves

The oligonucleotides **1** and **2** containing or not a single $[Pt(dien)(dG)]^{2+}$ adduct were allowed to anneal with the corresponding duplex **A** in 50 mM $NaClO_4$, 10 mM $Mg(ClO_4)_2$, 10 mM phosphate buffer pH 6.8. The triplex concentration was 1.5 μM. The mixture was heated at 50 °C for 10 min and then cooled overnight to 4 °C. The absorbances of the mixed oligonucleotides were measured at 260 nm as a function of temperature by means of a Kontron Uvikon 923 spectrophotometer. The temperature gradient was 0.15 °C min^{-1}. The melting temperature (T_m) was taken as the temperature corresponding to the maximum of the first derivative profile of the melting curves.

Conversion of *trans*-DDP monofunctional adducts

The third strands (Hoogsten strands) containing a single *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ or *trans*-$[Pt(NH_3)_2(dC)Cl]^+$ adduct, ^{32}P labelled at the 5′ end,[16] were allowed to anneal with their corresponding duplexes (1.5 μM) by incubation in 50 mM NaCl for 5 min at room temperature and then 30 min on ice. The triplexes were precipitated with ethanol and resuspended in 20 μl of 10 mM $Mg(ClO_4)_2$, 10 mM phosphate buffer pH 6.8 for triplexes $\mathbf{A.1}^t$ and $\mathbf{A.2}^t$ or in 20 μl of 150 mM $NaClO_4$, 5mM $Mg(ClO_4)_2$, 5 mM acetate buffer pH 5.0 for triplexes $\mathbf{B.3}^t$ and $\mathbf{B.4}^t$. The mixtures were then incubated at 25 °C or 37 °C. At various times aliquots were withdrawn, treated or not with thiourea to remove monofunctional adducts[26] and analysed by gel electrophoresis under denaturing conditions (24% polyacrylamide, 8 M urea). Quantitation of the gel bands was done on a Molecular Dynamics PhosphorImager using ImageQuant software (version 3.3) for data processing.

Acknowledgements

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