Fast Interstrand Cross-linking of Cisplatin – DNA Monoadducts Compared with Intrastrand Chelation: A Kinetic Study Using Hairpin-Stabilized Duplex Oligonucleotides

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Abstract: The antitumor drug cisplatin forms two kinds of guanine – guanine cross-links with DNA: intrastrand, occurring mainly at GG sites, and interstrand, formed at GC sites. The former are generally more abundant than the latter, at least in experiments with linear duplex DNA. The formation of interstrand cross-links requires partial disruption of the Watson – Crick base pairing, and one could therefore expect the cross-linking reaction to be rather slow. In contrast with this expectation, kinetic measurements reported here indicate that interstrand cross-linking is as fast as intrastrand, or even faster. We have investigated the reactions between two hairpin-stabilized DNA duplexes, containing either a $d(TGCA)_2$ sequence (duplex **TGCA**) or a $d(G^1G^2CA)$-$d(TG^3CC)$ sequence (duplex **GGCA**), and the diaqua form of cisplatin, *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$, in an unbuffered solution kept at pH 4.5 ± 0.1 and 20 °C. Using HPLC as the analytical method, we have determined the platination (first step) and chelation (second step) rate constants for these reaction systems. Duplex **TGCA**, in which the two guanines are quasi-equivalent, is found to be platinated very slowly ($k = 0.5 \pm 0.1\,\text{M}^{-1}\,\text{s}^{-1}$) and to form the final interstrand cross-link very rapidly ($k = 13 \pm 3 \times 10^{-3}\,\text{s}^{-1}$). For **GGCA**, we find that G^1 is platinated rapidly ($k = 32 \pm 5\,\text{M}^{-1}\,\text{s}^{-1}$) to form a long-lived monoadduct, which is only slowly chelated ($k = 0.039 \pm 0.001 \times 10^{-3}\,\text{s}^{-1}$) by G^2 (intrastrand), while G^2 is platinated one order of magnitude more slowly than G^1 ($k = 2.0 \pm 0.5\,\text{M}^{-1}\,\text{s}^{-1}$) and chelated fairly rapidly both by G^1 (intrastrand: $k = 0.4 \pm 0.1 \times 10^{-3}\,\text{s}^{-1}$) and G^3 (interstrand: $k = 0.2 \pm 0.1 \times 10^{-3}\,\text{s}^{-1}$); finally, G^3 is platinated at about the same rate as G^2 ($k = 2.4 \pm 0.5\,\text{M}^{-1}\,\text{s}^{-1}$) and chelated very rapidly by G^2 (interstrand: $k = 10 \pm 4 \times 10^{-3}\,\text{s}^{-1}$). These results suggest that the low occurrence of interstrand cross-links in cisplatinated DNA is due to an extremely slow initial platination of guanines involved in $d(GC)_2$ sequences, rather than to a slow cross-linking reaction.

Keywords: cisplatin · DNA · interstrand cross-links · kinetics · platinum

Introduction

The antitumor activity of cisplatin, *cis*-$[PtCl_2(NH_3)_2]$, is commonly ascribed to the 1,2-GG and 1,2-AG intrastrand cross-links that are formed as the major adducts when the drug reacts with DNA.[1] The kinetics of formation of intrastrand cross-links,[2–8] their structures,[9–11] and their recognition by HMG proteins, which are believed to be mediators of the anticancer activity,[12, 13] have been the subject of numerous studies. Conversely, the other type of cisplatin – DNA cross-links, the interstrand cross-links, have received somewhat less attention. Several factors have contributed to this situation. Firstly, interstrand cross-links are formed in smaller quantities, at least with linear DNA.[14, 15] Secondly, 1,2-GG and 1,2-AG intrastrand cross-links have been shown to display specific cytotoxicity in *E. coli*.[16, 17] Thirdly, in a clinical experiment, the levels of the 1,2-GG and 1,2-AG intrastrand cross-links in the white blood cells of cisplatin-treated patients were found to correlate with the response to the treatment.[18] Although these observations do suggest that the intrastrand cross-links may play a role in the cytotoxicity of cisplatin, they do not rule out involvement of interstrand cross-links. Interstrand cross-links are intrinsically more difficult to repair and therefore have a larger potential to kill cells, even if they are formed in smaller quantities. Interest in interstrand cross-links has recently been rekindled by the discovery of novel potent bi- and trinuclear platinum antitumor drugs, which are characterized by the increased levels of interstrand cross-links they form with DNA.[19]

One interesting aspect of the interstrand cross-links is their kinetics of formation. Experiments by Leng et al. have shown that double-stranded DNA bearing a *cis*-$\{PtCl(NH_3)_2\}^+$ group

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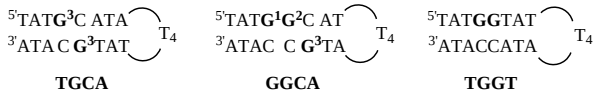
bound to the guanine of a d(AGC) trinucleotide converts quantitatively into the interstrand cross-link species between the $d(GC)_2$ guanines.[20] This indicates that the interstrand GG chelation of the aqua monoadduct (formed by hydrolysis of the chloro monoadduct) is considerably faster than the intrastrand $5' \rightarrow 3'$ AG chelation. Since the latter is only about 10 times slower than the intrastrand $5' \rightarrow 3'$ GG chelation,[6] one could predict that the interstrand cross-linking at a $d(GC)_2$ sequence should proceed at a rate comparable to that of the $5' \rightarrow 3'$ intrastrand GG chelation. This prediction was confirmed by work in this group (Reeder et al.[3]), in which we showed that, within the palindromic duplex d(TTGGCCAA)$_2$, the aqua monoadduct at the 3′-guanine formed the intra- and interstrand cross-links at comparable rates. These findings may appear surprising in view of the facts that the distance between the platinum atom of the monoadduct and the N7 atom of the chelating guanine is significantly larger for interstrand (∼7.5 Å) than for $5' \rightarrow 3'$ intrastrand (∼3.9 Å) cross-linking,[3] and that both guanosines of the $d(GC)_2$ sequence have to rotate about the sugar-phosphate chain in order to position the N7 atoms in the minor groove.[21–23] Furthermore, since the formation of the interstrand cross-link involves partial disruption of the Watson–Crick base pairing,[21–23] one may anticipate a significant influence of the base sequence in the vicinity of the $d(GC)_2$ site on the kinetics. Specifically, in the palindrome d(TTGGCCAA)$_2$, the duplex structure in the center is "locked" by the two GC pairs flanking $d(GC)_2$, which would be expected to disfavor interstrand cross-linking. By this reasoning, interstrand cross-linking within a $d(TGCA)_2$ sequence should proceed more rapidly. In order to check this hypothesis, and with the aim of gaining a deeper insight into the sequence-dependence of the kinetics of interstrand cross-linking, we investigated in this work the reaction between the diaqua form of cisplatin, *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$, and the two double-stranded oligonucleotides **TGCA** and **GGCA** shown below. In these, the



TGCA **GGCA** **TGGT**

tetranucleotides $d(TGCA)_2$ and d(GGCA)-d(TGCC) were placed in the center of hairpin-stabilized duplexes, respectively. The reactions were studied by the HPLC-based method developed in our laboratory.[24–26] All the platination and chelation rate constants were determined and compared to those reported previously for the palindrome d(TTGGCCAA)$_2$[3] and for the hairpin-duplex **TGGT**.[6] The results confirm our prediction and show that interstrand cross-linking is in fact very fast when the first platination occurs at a guanine within a TGC sequence.

Abstract in French: *Le complexe antitumoral cisplatine donne deux sortes d'adduits impliquant deux guanines comme ligands: les chélates intra-brin entre deux guanines adjacentes et les ponts inter-brins entre deux guanines de séquences $d(GC)_2$. Les premiers sont les plus abondants, au moins avec l'ADN duplex linéaire. La formation des ponts inter-brins requiert une dissociation locale de l'appariement de type Watson–Crick, ce qui laisse penser que la chélation du monoadduit est lente. Contrairement à cette prédiction, les résultats que nous présentons montrent que le pontage inter-brins est aussi rapide, voir plus rapide, que la chélation intra-brin. Nous avons étudié les réactions de deux duplex ADN en épingle à cheveux, contenant les séquences $d(TGCA)_2$ (duplex **TGCA**) et $d(G^1G^2CA)$-$d(TG^3CC)$ (duplex **GGCA**), avec le dérivé diaqua du cisplatine cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ en solution non tamponnée à pH 4.5 ± 0.1 et à 20 °C. Le suivi des réactions par chromatographie liquide à haute performance a permi de déterminer les constantes de vitesse des étapes de platination et de chélation. Le duplex **TGCA** a deux guanines pratiquement équivalentes platinées très lentement ($k = 0.5 \pm 0.1\ M^{-1}s^{-1}$), mais formant le pontage inter-brins final très rapidement ($k = 13 \pm 3 \times 10^{-3}\ s^{-1}$). Dans le cas de **GGCA**, les guanines sont différentes. G^1 est platinée rapidement ($k = 32 \pm 5\ M^{-1}s^{-1}$) et donne un monoadduit de longue durée de vie, transformé très lentement en chélate intra-brin avec G^2 ($k = 0.038 \pm 0.001 \times 10^{-3}\ s^{-1}$). En revanche, G^2 est platinée plus lentement que G^1 par un ordre de grandeur ($k = 2.0 \pm 0.5\ M^{-1}s^{-1}$) mais le monoadduit est chélaté rapidement à la fois en intra-brin par G^1 ($k = 0.4 \pm 0.1 \times 10^{-3}\ s^{-1}$) et en inter-brins par G^3 ($k = 0.2 \pm 0.1 \times 10^{-3}\ s^{-1}$). De plus, G^3 est platinée avec une vitesse voisine de G^2 ($k = 2.4 \pm 0.5\ M^{-1}s^{-1}$) mais le monoadduit est chélaté très rapidement en inter-brins ($k = 10 \pm 4 \times 10^{-3}\ s^{-1}$). Ces résultats suggèrent que la faible proportion d'adduits inter-brins formés lors de la réaction entre l'ADN et le cisplatine résulte d'une platination très lente des guanines impliquées dans la séquence $d(GC)_2$, plutôt que d'une éventuelle lenteur de la réaction de pontage inter-brins.*

Results and Discussion

In order to assess the duplex stability of **TGCA** and **GGCA** under our experimental conditions, the melting temperatures (at hairpin concentrations of 10^{-4} M in 0.1 M $NaClO_4$ at pH 4.4) were determined to be 53 and 55 °C, respectively. For **GGCA**, we also recorded NMR spectra of the imino region and CD spectra as functions of pH. Figures 1 and 2 show low-field ^{1}H NMR spectra and CD spectra of 10^{-4} M solutions of **GGCA** in 0.1 M $NaClO_4$, respectively, recorded at different pH values. In Figure 1 one can discern the peaks due to the imino protons of the GC ($\delta = 12.3 - 12.9$) and AT ($\delta = 13.1 - 13.5$) Watson–Crick base pairs, as well as those of the T_4 hairpin loop ($\delta = 10.4 - 11.2$). Evidently, at pH 4.4, the duplex stem is intact, and only one AT pair (probably the terminal pair) shows extensive H3 exchange. Figure 2 shows that **GGCA** has a conservative CD spectrum with a negative band at 250 nm and a positive band at 270 nm, typical for B-DNA.[27] Since it has been shown in previous studies on similar hairpins that, under the experimental conditions used here, monomolecular hairpin-duplex structures prevail over bimolecular associates,[28] we can conclude that both **TGCA** and **GGCA** are present in the form of the hairpin-duplexes shown above.

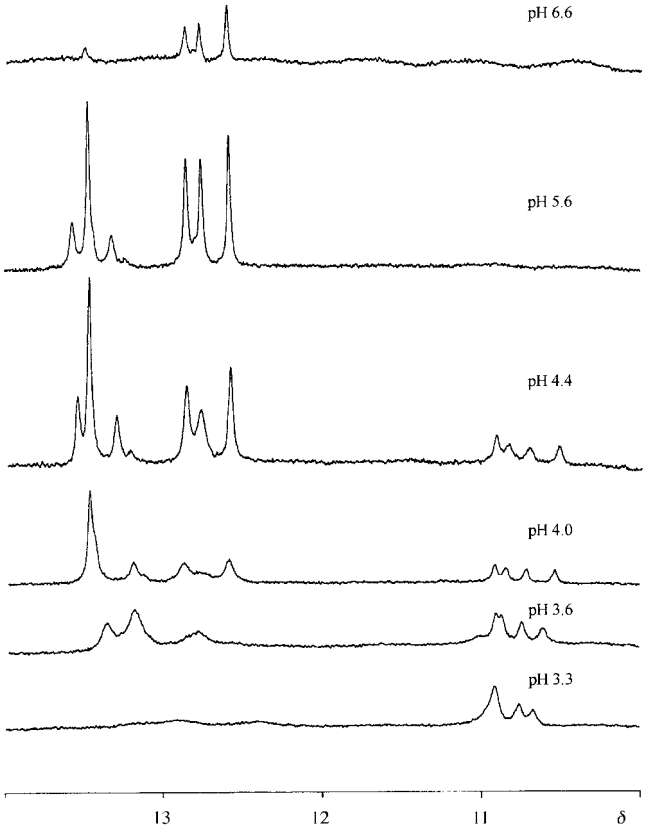


Figure 1. Imino region of ^{1}H NMR spectra recorded for 0.1 mM H_2O/D_2O (9:1) solutions of **GGCA** in 0.1M $NaClO_4$ at 20 °C.

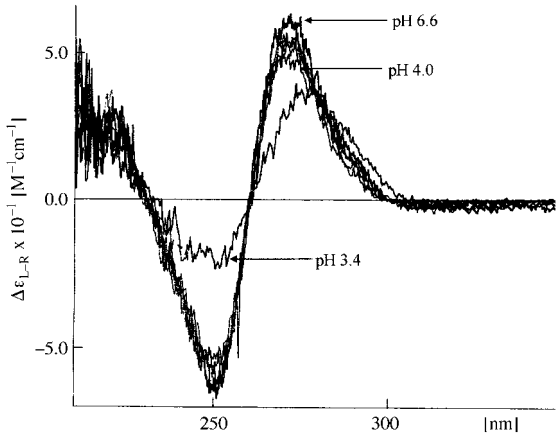


Figure 2. CD spectra recorded for 0.1 mM H_2O solutions of **GGCA** in 0.1M $NaClO_4$ at 20 °C, adjusted to pH values of 3.4, 4.0, 4.4, 5.6, and 6.6.

The two investigated reaction systems are shown in Schemes 1 and 2. Two typical examples of experimental and calculated concentration curves are given in Figure 3. The optimized rate constants are listed in Table 1. The guanines of **TGCA** were labeled G^3, since they have the same TGC context as G^3 in **GGCA**.

In **TGCA**, the two guanines are not exactly equivalent because of the asymmetry introduced by the hairpin; however, the duplex part $d(TATGCATA)_2$ is self-complementary. One may therefore expect almost identical platination and chelation rates for both reaction paths, and the concentration

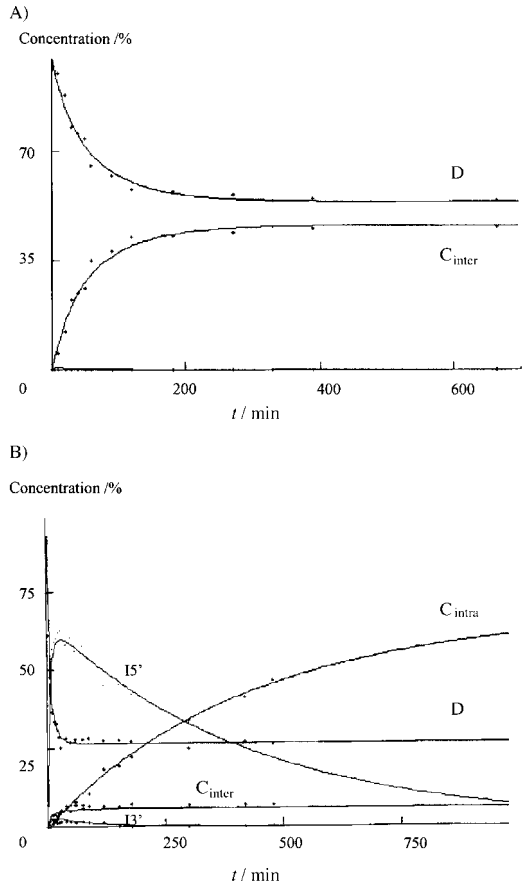


Figure 3. Experimental relative concentrations and calculated curves for two kinetic runs, A) between **TGCA** and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and B) between **GGCA** and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$. D: free oligonucleotides, I5′, I3′: monoadducts on G^1 and G^2, respectively, C_{intra}: intrastrand chelate, C_{inter}: interstrand cross-link.

curves were fitted under this assumption. Upon treatment of **TGCA** with *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$, we observed only peaks due to the starting oligonucleotide and the final interstrand cross-link. Even at short reaction times, no intermediate was detected. This is already an indication that the chelation reaction is very fast. Fitting to the experimental concentration curves yielded both rate constants with satisfactory precision. Whereas platination is slower than any platination reaction of a guanine within an oligonucleotide (single- or double-stranded) that we have ever measured under the same conditions, and slower even than the platination of Me-5′-dGMP$^-$ (1.18 ± 0.03 M^{-1}s^{-1}),[29] the chelation rate is the fastest of all Pt–DNA chelations that we have investigated so far. Both results are interesting in view of the reaction mechanism. The slow platination of **TGCA** as compared to Me-5′-dGMP$^-$ indicates that the environment of a DNA duplex can not only accelerate the association with a cationic metal complex (which is an expected effect of the favorable electrostatic interaction between the cation and the DNA polyanion) but may also have an inhibitory influence on the actual coordination step. The fast interstrand cross-linking in **TGCA** suggests that the low occurrence of interstrand cross-links in cisplatinated DNA may not be due to an unfavorable interstrand cross-linking rate, but rather to slow platination of $d(GC)_2$ sequences.

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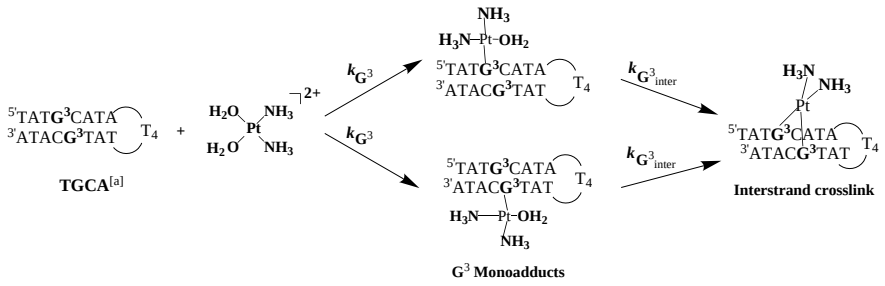


Scheme 1. Reaction system investigated for **TGCA**. [a] The guanines of **TGCA** were labeled G^3, as they have the same TGC environment as G^3 in **GGCA**.

The reaction between **GGCA** and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ involves three independent platination reactions and four chelation reactions, and is the most complicated reaction system that we have investigated so far. The HPLC chromatogram depicted in Figure 4 shows the well resolved peaks of six species: the starting oligonucleotide, three monoadducts, and the intrastrand and the interstrand cross-links. The chromatogram demonstrates the power of reverse-phase HPLC in resolving systems of very similar platinated oligonucleotides. The monoadduct on G^3 was observed only at the beginning of the reaction and in small quantities ($<2\,\%$), which prevented its precise quantification; however, its identification by the Maxam – Gilbert sequencing method (see Experimental Section) was unambiguous. That this monoadduct accumulates in such small quantities is due to the fast interstrand cross-linking, which is as fast as that of the monoadducts of **TGCA** (where the monoadducts were not observed at all). The optimization of the seven rate constants was a test of the robustness of the ITERAT program.[30] Since the starting concentration of the oligonucleotide (determination of which by UV spectrophotometry is rather imprecise) and the offset of the time scale were treated as additional variables, the optimization procedure in fact included nine variables. The concentration curve of the monoadduct on G^3 was not used in the optimization (since, as stated above, the concentrations could not be determined with sufficient precision). However, the corresponding theoretical curve was calculated and found to be in agreement with the small concentrations found experimentally. Full optimization of the nine parameters was achieved by successive block refinements (optimization of a limited number of variables while the others were kept fixed), increasing the number of free variables, and finally by releasing all of them. All the rate constants listed in Table 1 correspond to averages over three experiments and the estimated standard deviations show that all constants were determined fairly well. In any case, the differences between the individual rate constants that we discuss here by far exceed the experimental errors and their significance is thus beyond any doubt.

The rate constants given in Table 1 show that the platination of G^1 of **GGCA** is more than ten times faster than those of G^2 and G^3. The large difference between the platination rates for G^1 and G^2 is in agreement with the results that we reported earlier for the palindrome $d(TTGGCCAA)_2$.[3] Comparison of the platination rate constants for G^2 of **GGCA** ($2.0 \pm 0.5\ M^{-1}s^{-1}$) and for the 3′-G of **TGGT** ($15 \pm 1\ M^{-1}s^{-1}$),[6] together with the slow platination of **TGCA** ($0.5 \pm 0.1\ M^{-1}s^{-1}$), suggests that a

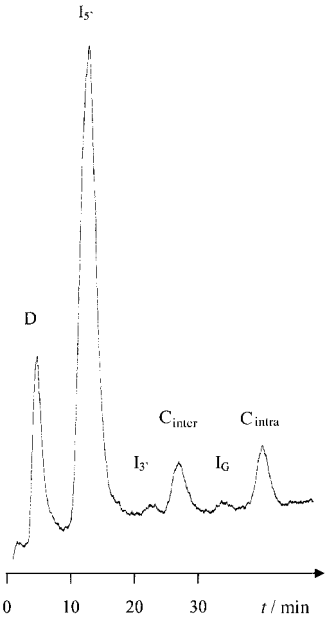
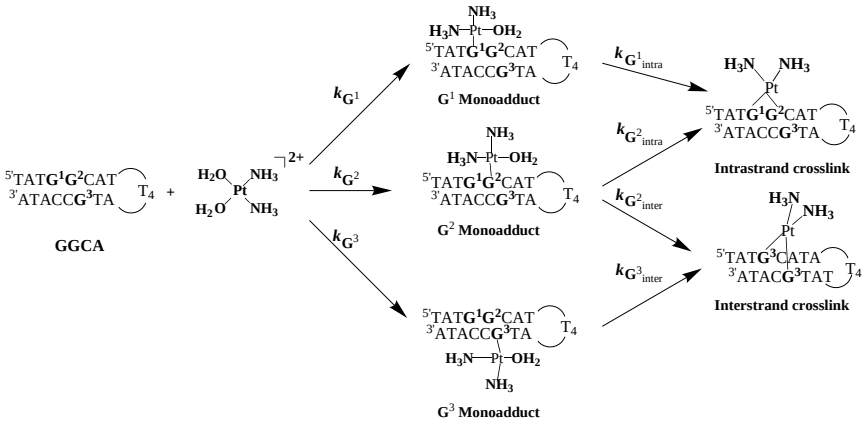


Figure 4. HPLC chromatogram for the reaction between **GGCA** and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ quenched by KBr after 40 min. Detection wavelength: 245 nm. Operating conditions are described in Experimental Section. D: free oligonucleotide, $I_{5'}$, $I_{3'}$, I_G: monoadducts on G^1, G^2, and G^3, respectively, C_{intra}: intrastrand chelate, C_{inter}: interstrand cross-link.



Scheme 2. Reaction system investigated for **GGCA**.

Table 1. Optimized rate constants for the reactions of oligonucleotides with *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ at pH 4.5 ± 0.1 and $T = 20\,^{\circ}C$ in 0.1 M $NaClO_4$.[a] Standard deviations are given in parentheses.

	Platination $[M^{-1}s^{-1}]$			Chelation $[\times 10^{-3}s^{-1}]$			
	$k_G{}^1$	$k_G{}^2$	$k_G{}^3$	$k_G{}^1{}_{intra}$	$k_G{}^2{}_{intra}$	$k_G{}^2{}_{inter}$	$k_G{}^3{}_{inter}$
5′TAT**G³**C ATA 3′ATAC **G³**TAT T4			0.5 (1)[c]				13 (2)
5′TAT**G¹G** C AT 3′ATAC C **G³**TA T4	32 (5)	2.0 (5)	2.4 (5)	0.039 (1)	0.4 (1)	0.2 (1)	10 (4)
d(TT**G¹G²**CCAA)$_2$[b]	27 (4)	2.2 (4)		0.06 (4)	0.8 (2)	0.4 (1)	

[a] Labeling of the rate constants is defined in Schemes 1 and 2. [b] Ref. [3]. [c] Individual rate constant for each **G³** guanine.

cytosine 3′ to a guanine slows down the platination of this guanine. On the other hand, a 5′-G seems to favor the platination of a given guanine,[6, 31] and the guanines of **TGCA** are therefore platinated more slowly than the G^2 of **GGCA**.

Since the G^1 monoadduct of **GGCA**, the most rapidly formed, is also chelated only very slowly, it attains a considerable concentration (up to 60% of all species) and is extremely long-lived (after 12 h, it still accounts for 10% of all species). Such monoadducts have the potential to form cross-links with recognition proteins, and we have already raised the question of whether they could play a role in the cytotoxicity of cisplatin.[32] Whereas G^2 and G^3 are platinated with comparable rates, the chelation rates of their monoadducts differ significantly, the interstrand cross-linking of G^3 being more than one order of magnitude faster than both (intrastrand and interstrand) chelation reactions of G^2.

The 50-fold difference between the two interstrand cross-linking rates determined for **GGCA** is particularly intriguing, as both reactions yield the same product. This points to a significant kinetic difference in the two bridging pathways. As the G^2 monoadduct is flanked by GC pairs on both sides, one would be tempted to relate its slower interstrand cross-linking to stronger stacking limiting its mobility. However, recent work by Fritzsche et al. has shown that flanking GC pairs can have the opposite effect, accelerating the base-pair opening.[33] Interestingly, the GC base-pair lifetime of an $(AGCT)_2$ sequence within a duplex dodecamer (7 ± 4 ms at 15 °C) was found to be considerably shorter than that of the inverse sequence $(TCGA)_2$ (40 ± 10 ms at 15 °C).[34] The fast interstrand cross-linking observed in our work could thus be related to the fast opening rates of GC pairs involved in $(GC)_2$ sequences (although it remains to be shown that GC pairs in a $(TGCA)_2$ sequence open as rapidly as in an $(AGCT)_2$ sequence). A rough estimation of the rate constant for the successive opening of two base pairs suggests that this may indeed be the rate-limiting step for the interstrand cross-link formation. From the GC base-pair lifetime of 7 ms determined for the $(AGCT)_2$ sequence at 15 °C, we obtain a rate constant for base-pair opening k_{op} of $\sim 150\ s^{-1}$, and the lifetime of an opened pair of ~ 10 ns yields a closing rate constant k_{cl} of $\sim 10^8\ s^{-1}$.[34] Then, since $k_{cl} \gg k_{op}$, the successive opening of two GC base pairs should occur—assuming no cooperativity—with a rate constant $k_{op,2} \approx k_{op}^2/k_{cl} \approx 2 \times 10^{-4}\ s^{-1}$ at 15 °C. This is more than one order of magnitude slower than the interstand cross-linking rate observed (at 20 °C) for the G^3 monoadduct; however, for a guanine bearing the platinum monoadduct, the opening might be faster. Moreover, cooperative effects could accelerate the opening of the second base pair. Therefore, the hypothesis that the successive opening of the two base pairs of a $(GC)_2$ sequence bearing a platinum monoadduct determines the rate of interstrand cross-linking seems reasonable.

The steps following the opening of the two base pairs must include a rotation of the two guanines about the sugar-phosphate backbone, as apparent from NMR and X-ray structures of the final interstrand cross-link.[21–23] A strikingly similar structure featuring the guanine N7 atoms in the minor groove has been reported for the dodecamer d(CGCGAGTTCGCG)$_2$, from which the two central mismatched thymines were excised by the mismatch DNA glycosylase.[35] The fact that this rearrangement of the guanines is favorable when the complementary bases are absent suggests that it could also occur spontaneously in a $(GC)_2$ sequence where the cytosines are extruded from the helix. Cytosine extrusion itself is a feasible process, as demonstrated recently by the X-ray structure of the complex formed between the HhaI methyltransferase and its recognition sequence 5′-GCGC-3′.[36] This crystal structure shows the 3′-cytosine swung out and extruded out of the DNA helix, and bound to the active site. Although these crystal structures do not provide any information on the kinetics of the rearrangements involved, they do allow a conceivable three-step interstrand cross-linking mechanism to be formulated: 1) initial (and probably rate-limiting) successive opening of the two GC base pairs with extrusion of the cytosines, 2) subsequent rotation and restacking of the guanines, positioning the platinum atom of the monoadduct in proximity to the N7 atom of the cross-linking guanine, and 3) ligand substitution.

Conclusion

This work has revealed two new kinetic aspects of cisplatin–DNA interstrand cross-linking at $d(GC)_2$ sequences. Firstly, the guanines involved in these sequences are shown to react particularly slowly with the diaqua form of cisplatin, apparently reflecting an inhibitory effect of the 3′-cytosine upon binding platinum. Secondly, the formation of interstrand cross-links from the aqua monoadducts bound to a guanine within a $d(GC)_2$ sequence is relatively fast. Its rate is strongly dependent on the base preceding the monoplatinated G* on the 5′-side: a G* monoadduct within a (TG*C)(GCA) sequence forms the interstrand cross-link with a rate of $\sim 10^{-2}\ s^{-1}$ at 20 °C, whereas the interstrand cross-linking within a (GG*C)(GCC) sequence is ~50 times slower. Interestingly, we did not detect any significant influence due to the 5′-base adjacent to the cross-linking guanine: G* monoadducts within (TG*CA)(TGCA) and (TG*CC)-(GGCA) sequences formed interstrand cross-links with similar rates.

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These results indicate that the low yield of interstrand cross-links observed upon treatment of linear duplex DNA with cisplatin[14, 15] is due to slow platination at $d(GC)_2$ sites rather than to slow interstrand cross-linking. Furthermore, they suggest that the influence of DNA supercoiling on the yield of interstrand cross-links[37] could be due to enhancement of the reactivity of the $d(GC)_2$ guanines rather than to favoring of the cross-linking reaction (which should be fast anyway). It is conceivable that supercoiling diminishes the inhibitory effect that the 3′-C exerts upon platination of a given guanine.

Experimental Section

Starting materials: The oligonucleotides were synthesized as their ammonium salts by the phosphodiester method, by the group of Dr. T. Huynh Dinh (Institut Pasteur, Paris), and their purities were checked by capillary electrophoresis and mass spectroscopy. Approximate concentrations were evaluated photometrically from molar absorption coefficients $\varepsilon_{260\,nm} = 8000\,M^{-1}cm^{-1}$ per base. Cisplatin was provided by Johnson Matthey. Solutions of *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ were prepared by dissolving *cis*-$[Pt(NO_3)_2(NH_3)_2]$[38] in water. Perchloric acid was purchased from Merck (Germany), all salts (of HPLC grade) were purchased from Acros Organics (France). The 3′-exonuclease venom phosphodiesterase (VPD) was obtained from Boehringer – Mannheim (Germany).

Sample preparation for HPLC studies: The reactions were carried out in 0.1M $NaClO_4$ at 20 °C, pH 4.5 ± 0.1 adjusted by addition of $HClO_4$ or NaOH solutions (0.1M). The initial concentrations were $(1.2 - 1.5) \times 10^{-4}$M for the oligonucleotides and $(0.8 - 1.0) \times 10^{-4}$M for the diaqua complex. Aliquots were collected at several time points, quenched by additions of saturated KBr or KCl solutions, and stored at liquid nitrogen temperature until they were analyzed.[39]

HPLC analysis: HPLC analysis of the reaction aliquots was performed with a Beckman 126 pump equipped with a Rheodyn 7725 valve, coupled to a Beckman 166 diode array detector and a System Gold V810 integrator.

Reaction between GGCA and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$: Quenching with saturated KBr solution; stationary phase, Poros R_2H column (Perspective Biosystems GmbH, France); mobile phase, ammonium bromide (1M), triethylammonium acetate (0.02M); acetonitrile gradient, 3% for 5 min, then 3 – 6% in 50 min; flow rate 3 mL min^{-1}; column temperature 40 °C.

Reaction between TGCA and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$: Quenching with saturated KCl solution; stationary phase, Nucleosil C8 (150 × 4.6 mm, 5 μm, 300 Å) stainless steel column (Colochrom, France); mobile phase, ammonium chloride (0.5M), triethylammonium acetate (0.02M); acetonitrile, gradient, 9% for 5 min, then 9 – 10% in 7 min, flow rate 1 mL min^{-1}; column temperature 40 °C.

Relative concentrations were determined from the ratios of the peak areas. The detection wavelength of 245 nm was chosen as it is close to the isosbestic points of the reactions. The reaction intermediates were identified either by enzymatic digestion of the products followed by MALDI mass spectrometric analysis of the isolated fragments, as previously described,[39] or by a Maxam – Gilbert sequencing experiment to determine the platinum-binding site, as described by Redon et al.[40] The rate constants were calculated by numerical integration of the differential equations, using the ITERAT program.[30]

Spectroscopic studies: The concentrations of the oligonucleotides and the melting profiles were determined on a Uvicon 941 spectrophotometer. The CD spectra were recorded on a Jobin Yvon Mark IV dichrograph. The NMR spectra were recorded on a Bruker 500 MHz spectrometer, with a 1-3-3-1 pulse sequence used to suppress the H_2O signal.[41]

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