Walking of antitumor bifunctional trinuclear Pt^{II} complex on double-helical DNA

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ABSTRACT

The trinuclear BBR3464 ([{*trans*-PtCl(NH$_3$)$_2$}$_2$μ-(*trans*-Pt(NH$_3$)$_2$(H$_2$N(CH$_2$)$_6$NH$_2$)$_2$)]$^{4+}$) belongs to the polynuclear class of platinum-based anticancer agents. DNA adducts of this complex differ significantly in structure and type from those of clinically used mononuclear platinum complexes, especially, long-range (Pt, Pt) intrastrand and interstrand cross-links are formed in both 5′–5′ and 3′–3′ orientations. We show employing short oligonucleotide duplexes containing single, site-specific cross-links of BBR3464 and gel electrophoresis that in contrast to major DNA adducts of clinically used platinum complexes, under physiological conditions the coordination bonds between platinum and N7 of G residues involved in the cross-links of BBR3464 can be cleaved. This cleavage may lead to the linkage isomerization reactions between this metallodrug and double-helical DNA. Differential scanning calorimetry of duplexes containing single, site-specific cross-links of BBR3464 reveals that one of the driving forces that leads to the lability of DNA cross-links of this metallodrug is a difference between the thermodynamic destabilization induced by the cross-link and by the adduct into which it could isomerize. The rearrangements may proceed in the way that cross-links originally formed in one strand of DNA can spontaneously translocate from one DNA strand to its complementary counterpart, which may evoke walking of the platinum complex on DNA molecule.

INTRODUCTION

DNA binding, and the consequences for structure and function, is the mechanistic paradigm by which many drugs exert their antitumor activity (1). Structural changes are manifested in many ways, both through covalent modification (the alkylating and platinating agents), 'non-covalent' intercalators and minor-groove binders as well as strand breakage. However, DNA as a template affects kinetics of substitution reactions occurring within its domain by exerting steric and electronic effects on small molecules interacting with it (2). The nature of DNA—single- versus double stranded—may also affect kinetics of aquation of platinum-based antitumor agents (3). Most studies of DNA adducts involve either global modification or site-specific modification where the consequences of structural change and protein recognition may be examined. The dynamic nature of these DNA adducts, which may be dependent on the nature of their structural distortions, has been less well examined.

Platinum coordination compounds represent excellent substrates for study of adduct interchange because, apart from their clinical relevance, steric effects, geometry and comparison of monofunctional or bifunctional substitution on the DNA template may be systematically explored. Indeed, for a series of mononuclear Pt^{II} complexes, adducts formed in the reaction between these complexes and DNA have been shown to be susceptible to further transformations (4–7). The coordination bonds between platinum and the base residues in bifunctional adducts can be spontaneously cleaved with essentially one cleavage reaction per cross-link (CL). Such a cleavage generates intermediate monofunctional adducts which can then react further to either restore the original CL or form new structures, which may be DNA–DNA or even DNA–protein CLs (7,8).

The trinuclear Pt^{II} complex, BBR3464 ([{*trans*-PtCl(NH$_3$)$_2$}$_2$μ-(*trans*-Pt(NH$_3$)$_2$(H$_2$N(CH$_2$)$_6$NH$_2$)$_2$)]$^{4+}$), is one example of the polynuclear class of platinum drugs in which the platinum coordination units are linked by alkanediamine chains (9). This complex was designed on the basis of the hypothesis systematically tested

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by us and others that structurally novel platinum complexes that bind to DNA differently than conventional antitumor mononuclear cisplatin {*cis*-[PtCl$_2$(NH$_3$)$_2$], *cis*-diamminedichloridoplatinum(II)} may have distinct cytotoxicity and side effect profiles (10). This hypothesis stems from the fact that the antitumor activity of platinum-based drugs is mediated by their ability to attack DNA coordinating preferentially to guanine residues (11,12).

Biochemical and molecular biophysics experiments established that BBR3464 forms a global DNA adduct profile which is fundamentally different from that of cisplatin. While the most common adduct formed by reaction of cisplatin with DNA is a short-range 1,2-intrastrand CL formed between neighboring purine residues (~90%), the trinuclear BBR3464 binds DNA in a way very different from cisplatin. The distance between Pt–Cl units in BBR3464 is ~2.5 nm (13,14), giving the drug the ability to form long-range interstrand and intrastrand CLs, where the platinated nucleotides are separated by several (up to four) intervening base pairs (15,16). As far as long-range interstrand CLs are concerned, BBR3464 forms these lesions in natural DNA in a considerably higher amount (~20%) (15) than cisplatin not only in the 5′–5′ direction like cisplatin, but also in the less usual 3′–3′ direction (17). Interestingly, the directionality of interstrand CLs of BBR3464 is dependent on the nature of the CL. The 1,2-interstrand CL forms in only the 3′–3′ direction and the 1,4-interstrand CL is formed in both directions, while the 1,6-interstrand CL forms in only the 5′–5′ direction (17). The properties of these site-specific adducts of BBR3464, such as conformational distortions, are also distinctly different from those of the short-range adducts of mononuclear cisplatin (17,18).

Importantly, in studying the properties of site-specific long-range interstrand CLs of bifunctional polynuclear Pt^{II} complexes, rearrangement of a small proportion into intrastrand CLs can occur (19). These possible transformations may be distinguished from the mononuclear cases above because the latter involves bifunctional adducts on one Pt center whereas in the polynuclear case two monofunctional sites are involved. Thus, the inherent steric effects around the Pt center of the mononuclear bifunctional adduct are replaced by the steric constraints of the conformational change as a whole. Since specific adducts of BBR3464 distort DNA conformation differently, it is reasonable to expect that the energetic signatures of these dissimilar adducts are different. Hence, in the present work we focused on establishing correlations between the energetic impact of various DNA adducts of BBR3464 and their susceptibility to further transformation. The results show for the first time that the adduct formed on one strand of DNA can spontaneously translocate to its complementary strand.

MATERIALS AND METHODS

Starting materials

The nitrate salt of BBR3464 (Figure 1A) was prepared as described elsewhere (16,20). The stock solution of BBR3464 was prepared at the concentration of 5×10^{-4} M

in MilliQ H$_2$O and stored at 4°C in the dark. The concentration of platinum in the stock solution was determined by flameless atomic absorption spectrometry (FAAS). The synthetic oligodeoxyribonucleotides were purchased from VBC-Genomics (Vienna, Austria) and purified as described earlier (21,22). T4 polynucleotide kinase was from New England Biolabs (Beverly, MA, USA). Acrylamide, bis(acrylamide), urea and NaCN were from Merck KgaA (Darmstadt, Germany). DMS was from Sigma (Prague, Czech Republic). [γ-^{32}P]ATP was from Amersham (Arlington Heights, IL, USA).

Platinations of oligonucleotides

Preparation of the intrastrand CL. The oligonucleotide duplexes containing single, site-specific intrastrand CLs of BBR3464 in the top strands of the duplexes shown in Figure 1B and C were prepared as described earlier (18) (see also Supplementary data SI).

Preparation of the interstrand CL. The oligonucleotide duplexes containing single, site-specific interstrand CLs of BBR3464 shown in Figure 1C were also prepared as described earlier (17) (see also Supplementary data SI).

Kinetics of isomerization

The platinated top strands of the duplexes shown in Figure 1B and C containing a single, site-specific intrastrand CL of BBR3464 (5′-end-labeled or non-labeled) were mixed with unplatinated complementary bottom strands (non-labeled or 5′-end-labeled, respectively) in 10 mM Tris–Cl (pH 7.4) and 100 mM or 150 mM NaClO$_4$ and incubated at 37°C. Aliquots were withdrawn at various time intervals, the products were separated on denaturing 24% PAA/8 M urea gel (PAA = polyacrylamide) and the bands were analyzed by densitometry.
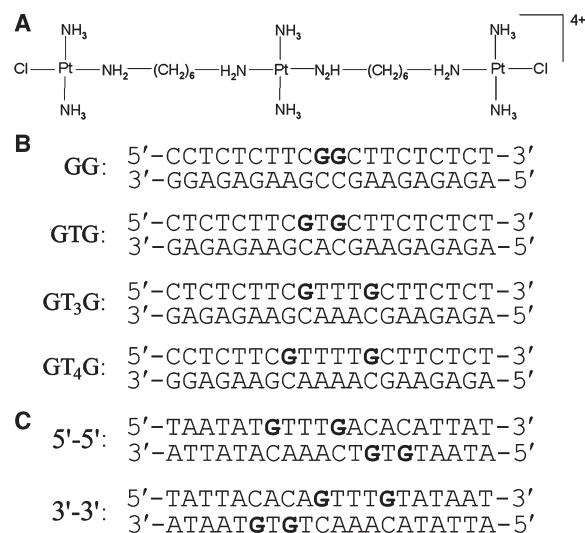

Figure 1. (**A**) Structure of BBR3464. (**B**) Nucleotide sequences of the 20-bp oligodeoxyribonucleotide duplexes GG, GTG, GT3G and GT4G. (**C**) Nucleotide sequences of the 20-bp oligodeoxyribonucleotide duplexes 5′–5′ and 3′–3′.

Differential scanning calorimetry

Excess heat capacity (ΔC_p) versus temperature profiles for the thermally induced transitions of duplexes were measured using a VP-DSC Calorimeter (Microcal, Northampton, MA, USA). Other details were the same as in our previous articles (23–25) (see also Supplementary data SI).

RESULTS AND DISCUSSION

Rearrangement of the intrastrand CL formed by BBR3464 in double-helical DNA

We demonstrated in our previous articles (15,17,18) that preferential G binding of BBR3464 results in various types of adducts including long range intrastrand and interstrand CLs. Considering this fact we have designed a series of synthetic oligodeoxyribonucleotide duplexes, GG, GTG, GT$_3$G and GT$_4$G (Figure 1B). The pyrimidine-rich top strands of these duplexes only contained two G residues in the center (Figure 1B, bold). These top strands were modified by BBR3464 so that they contained a single 1,2-, 1,3-, 1,5-, or 1,6-intrastrand CL of this platinum complex between two G residues separated by 0, 1, 3 or 4 nt, respectively.

The top strands of the duplexes GG, GTG, GT$_3$G and GT$_4$G (5′-end labeled) containing the intrastrand CL were hybridized with their complementary bottom strands (which were not radioactively labeled) for 2 h at 4°C in the buffer containing 10 mM Tris–Cl (pH 7.4) and 100 mM NaClO$_4$. Thus, the samples of these duplexes (20 μM) were prepared so that each contained a single, site-specific intrastrand CL between the two G residues in the top strand and only the top strand of these duplexes was 5′-end-labeled. These samples were further incubated at 37°C. At various time intervals, aliquots were withdrawn and analyzed by gel electrophoresis under denaturing conditions. The intrastrand CLs formed by BBR3464 after pairing the platinated single-stranded oligonucleotides with their complementary strands were labile except the 1,3-GTG intrastrand CL. As a function of time, the radioactivity associated with the bands corresponding to the top strands of the duplexes containing either 1,2-, 1,5- or 1,6-intrastrand CL of BBR3464 decreased with a concomitant appearance of a new, markedly more slowly migrating species (shown for GT$_3$G duplex in Figure 2A); the radioactivity associated with this new species increased with growing time of the incubation. An explanation of this observation is transformation of the intrastrand CL resulting in the interstrand cross-linked duplex (26). In addition, if these markedly more slowly migrating species were cut-off from the gels, eluted and purified in the same way as described in previous articles (17,27) and subsequently platinum was removed from these products after its incubation with 0.2 M NaCN (pH 11) at 45°C for 10 h in the dark, the resulting products comigrated in the gel with the unplatinated top strands (shown for GT$_3$G duplex in Supplementary Figure S1). These results were interpreted to mean that the 1,2-, 1,5- or 1,6-intrastrand CLs were transformed into an interstrand CL whereas the
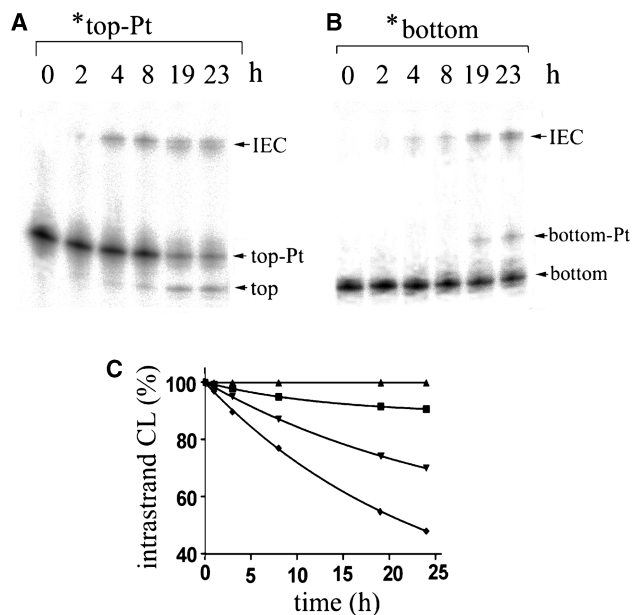

Figure 2. Rearrangement of the intrastrand CL formed by BBR3464 in the 20-bp duplexes GG, GTG, GT$_3$G and GT$_4$G. The top strands of these duplexes were modified so that they contained a single, site-specific 1,2-GG, 1,3-GTG, 1,5-GT$_3$G and 1,6-GT$_4$G intrastrand CL, respectively. These platinated single-stranded oligodeoxyribonucleotides (20 μM) were allowed to anneal with the unplatinated complementary (bottom) strands in 10 mM Tris–Cl (pH 7.4) and 100 mM NaClO$_4$ and further incubated at 37°C. At various time intervals, the aliquots were withdrawn and analyzed by electrophoresis in 12% PAA/8 M urea gel. Autoradiograms of the gels of the duplex GT$_3$G modified by BBR3464 radioactively labeled at the 5′-end of its top (**A**) or bottom (**B**) strand. Incubation times are indicated above each lane. (**C**) Plot of the percentages of intrastrand CLs of BBR3464 in the 20-bp duplexes GG (filled square), GTG (filled triangle), GT$_3$G (filled inverted triangle) and GT$_4$G (filled rhombus) versus time. These percentages were calculated from the ratio of the radioactivity in each lane associated with the band corresponding to the platinated top strand to the sum of the radioactivities associated with all bands (multiplied by 100). For other details, see the text.

1,3-intrastrand CL was stable not only in the single-stranded oligonucleotide, but also in the duplex. After 24 h of incubation of the duplexes GG, GT$_3$G and GT$_4$G containing an intrastrand CL of BBR3464, the amount of these CLs transformed into the interstrand CL varied and was dependent on the length of the intrastrand CL (Figure 2C). The trend, as the rate of this rearrangement reaction is concerned, was 1,6- >1,5- >1,2-intrastrand CL.

A careful examination of the gels used to analyze the duplexes in which the top strands were only 5′-end-labeled with ^{32}P also revealed a faint band migrating slightly, but significantly faster than the bands corresponding to the modified top strands (shown for GT$_3$G duplex in Figure 2A). These faint bands appeared only at longer incubation times (∼8 h) and their intensity increased with the incubation time. The species contained in this new faint band migrated at approximately the same rate as the unplatinated top 20-mer single strands of duplexes GG, GT$_3$G and GT$_4$G (shown in Figure 2A for the duplex GT$_3$G). The products contained in these faint bands were cut-off from the gels, eluted and purified and the amount

of Pt atoms contained in these products were estimated by FAAS and absorption spectrophotometry. The results confirmed that these products contained no platinum.

The identical experiments were also performed with the duplexes GG, GTG, GT$_3$G and GT$_4$G in which the bottom strand was only 5′-end-labeled with ^{32}P. In principle, the results obtained were analogous to those with the duplexes in which their top strands were radioactively labeled. As a function of time, the radioactivity associated with the band corresponding to the bottom strand of the duplex GTG containing 1,3-intrastrand CL remained unaffected. In contrast, the radioactivity associated with the bands corresponding to the bottom strands of the duplexes GG, GT$_3$G and GT$_4$G containing either 1,2-, 1,5- or 1,6-intrastrand CL of BBR3464, respectively, decreased with a concomitant increase of the radioactivity associated with the new, markedly more slowly migrating species corresponding to the interstrand cross-linked duplexes (shown for GT$_3$G duplex in Figure 2B). The platinated bases in the bottom strands of the interstrand cross-linked duplexes resulting from the rearrangement of the intrastrand CLs in the top strands of the duplexes were impossible to identify unambiguously since the bottom strands of these duplexes contained several Gs as potential sites involved in these interstrand CLs. Nevertheless, Maxam–Gilbert [dimethyl sulfate (DMS)] footprinting of the markedly more slowly migrating species revealed that the rearrangement of the intrastrand CL in the top strand led to the reduction of the intensity of the bands corresponding to several G's in the bottom strand (shown for GT$_3$G duplex in Supplementary Figure S2). This result suggests that these species emergent from rearrangement of intrastrand CLs were various long-range interstrand CLs of BBR3464.

Moreover, another new (faint) bands migrating slightly, but significantly more slowly than the bands corresponding to the unplatinated bottom strands (shown for GT$_3$G duplex in Figure 2B) appeared. This new species comigrated with the bottom single strands containing an intrastrand CL. The amount of Pt atoms contained in these products was estimated by FAAS and absorption spectrophotometry. The results confirmed that the ratio of Pt:nucleotide in these products was 3:20; this result is consistent with thesis that one molecule of the trinuclear Pt complex BBR3464 was bound to one molecule of the 20-mer bottom strand.

In aggregate, our results are consistent with the view and strongly support the thesis that some intrastrand and interstrand CLs formed in double-helical DNA by BBR3464 are susceptible to intramolecular rearrangement. More specifically, our results demonstrate that BBR3464 was translocated from originally platinated top strand to originally unplatinated bottom strand via intermediate interstrand CL.

In order to further explore this phenomenon we have designed two synthetic 20-bp oligodeoxyribonucleotide duplexes, 5′–5′ and 3′–3′, whose sequences are shown in Figure 1C. The pyrimidine-rich top strands of these duplexes only contained two G residues in the sequence GT$_3$G in the center (Figure 1C, bold). These top strands were modified by BBR3464 so that they contained a single 1,5-intrastrand CL of this platinum complex between the two Gs. The duplexes 5′–5′ and 3′–3′ were also designed in such a way that their bottom (complementary) strands contained only two Gs (shown in boldface in Figure 1C). Thus, given that the 1,5-intrastrand CL in the top strand is susceptible to rearrange into a G(N$_n$)G interstrand CL (N is a nucleotide and $n = 0,1,3,4$) and that BBR3464 tends to form long-range CLs spanning maximum 6 nts (up to a 1,6-GG CL) (15,16) then the 5′ G of the 1,5-intrastrand CL of BBR3464 in the top strand of the duplex 5′–5′ might rearrange into the 1,3- or 1,5-interstrand CL in the 5′–5′ orientation involving 3′ or 5′ G, respectively, in the bottom strand. Similarly, the 3′ G of the 1,5-intrastrand CL of BBR3464 in the top strand of the duplex 3′–3′ might rearrange into the 1,3- or 1,5-interstrand CL in the 3′–3′ orientation involving 5′ or 3′ G, respectively, in the bottom strand.

The duplex 5′–5′ containing the single, site-specific 1,5-intrastrand CL in which only the bottom strand was 5′-end labeled was incubated at 37°C in the medium of 10 mM Tris–Cl (pH 7.4) and 150 mM NaClO$_4$ and analyzed by gel electrophoresis. As a function of time, the radioactivity associated with the band corresponding to the bottom strand of this duplex 5′–5′ containing 1,5-intrastrand CL of BBR3464 decreased with a concomitant increase of the radioactivity associated with the new, markedly more slowly migrating species [Figure 3A (right) and C]. This result demonstrates that the 1,5-intrastrand CLs in the duplex 5′–5′ was transformed into an interstrand CL. The platinated bases in the product resulting from the rearrangement of the intrastrand cross-linked duplexes were identified from Maxam–Gilbert (DMS) footprinting. The result confirmed that the product was an interstrand CL formed between the 3′ G of the top strand and the 3′ G in the bottom strand of the duplex 5′–5′, i.e. the 1,3-interstrand CL was formed in the 5′–5′ direction. In addition, a faint band occurred migrating slightly, but significantly more slowly than the band corresponding to the nonmodified bottom strand. This faint band appeared only at longer incubation times (∼1 day) and the species corresponding to this band migrated in the gel at the same rate as the bottom single strand of the duplex 5′–5′ (Figure 3A, right) containing single, site-specific 1,3-GTG intrastrand CL of BBR3464. It was also verified by platinum FAAS and by the measurements of the optical density that the product contained three platinum atoms per one molecule of this product. Hence, a plausible explanations of these results is that 1,5-intrastrand CL of BBR3464 in the top strand of the duplex 5′–5′ isomerizes forming successively first 1,3-interstrand CL in the 5′–5′ direction which further isomerizes forming 1,3-intrastrand CL in the bottom strand.

The identical experiments were also performed with the duplex 5′–5′ in which the top strand (containing 1,5-intrastrand CL of BBR3464) was only 5′-end-labeled with ^{32}P. The radioactivity associated with the band corresponding to the top strand of the duplex 5′–5′ containing 1,5-intrastrand CL decreased with a concomitant increase of the radioactivity associated with the new species
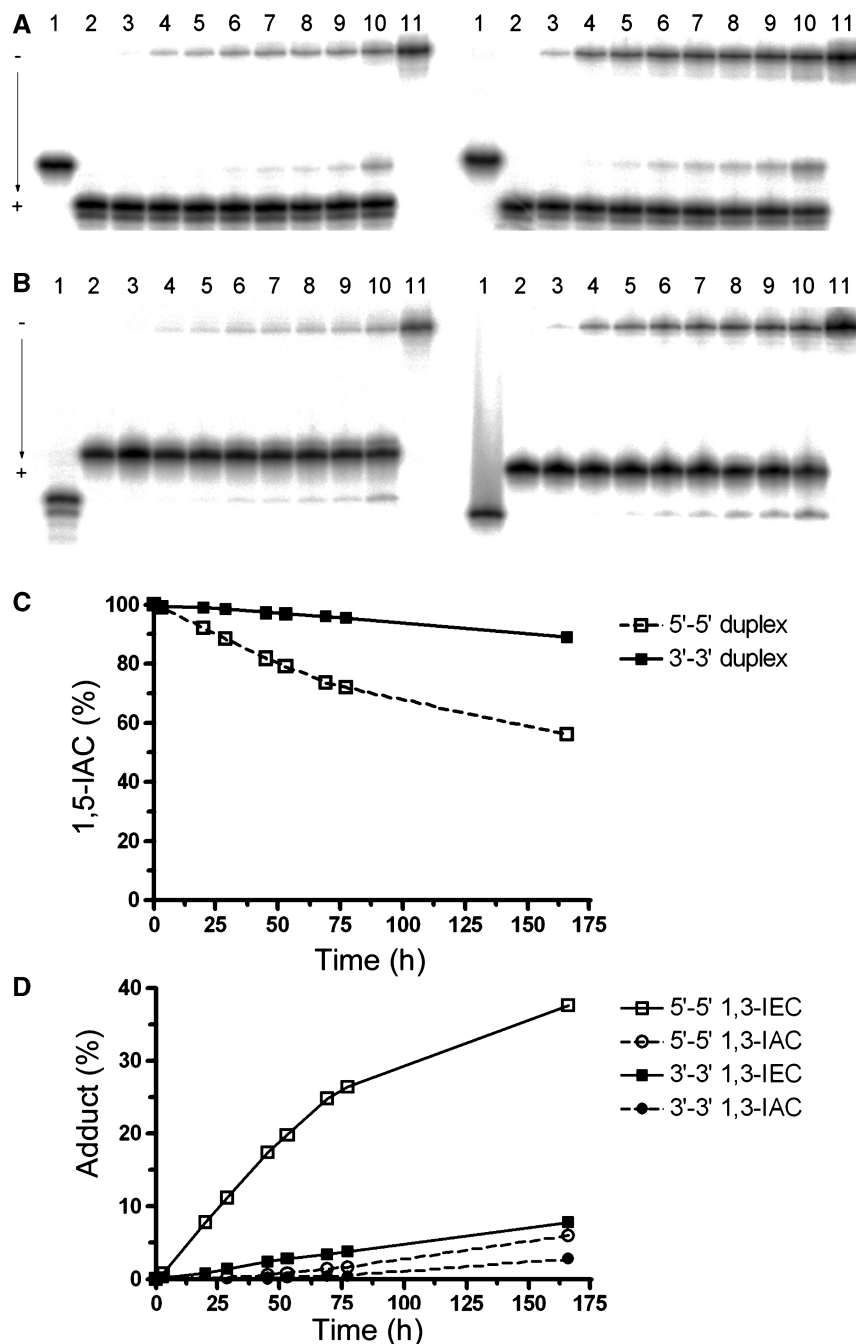
Figure 3. Rearrangement of the 1,5-intrastrand CL formed by BBR3464 in the 20-bp duplexes 5′–5′ and 3′–3′. The top strands of these duplexes were modified so that they contained a single, site-specific 1,5-GT$_3$G-intrastrand CL. These platinated single-stranded oligodeoxyribonucleotides (20 μM) were allowed to anneal with the unplatinated complementary (bottom) strands in 10 mM Tris–Cl (pH 7.4) and 150 mM NaClO$_4$. At various time intervals, the aliquots were withdrawn and analyzed by electrophoresis in 24% PAA/8 M urea gel. (**A**) Autoradiograms of the gels of the duplex 5′–5′ (right) and 3′–3′ (left) modified by BBR3464 radioactively labeled at the 5′-end of its bottom strand. Lane 1, 5′-end labeled single-stranded bottom strand containing 1,3-intrastrand CL of BBR3464; lanes 2–10, duplex incubated at 37°C for 0, 3, 20, 29, 45, 53, 69, 77 and 166 h, respectively; lane 11, 5′-end labeled duplex containing 1,3-interstrand CL of BBR3464. (**B**) Autoradiograms of the gels of the duplex 5′–5′ (right) and 3′–3′ (left) modified by BBR3464 radioactively labeled at the 5′-end of its top strand containing 1,5-intrastrand CL of BBR3464. Lane 1, 5′-end labeled single-stranded top strand; lanes 2–10, duplex incubated at 37°C for 0, 3, 20, 29, 45, 53, 69, 77 and 166 h, respectively; lane 11, 5′-end labeled duplex containing 1,3-interstrand CL of BBR3464. (**C**) Plot of the percentages of 1,5-intrastrand CL of BBR3464 [1,5-IAC (%)] in the 20-bp duplexes 5′–5′ (open square) and 3′–3′ (filled square) versus time. These percentages were calculated from the ratio of the radioactivity in each lane associated with the band corresponding to the nonmodified bottom strand to the sum of the radioactivities associated with all bands (multiplied by 100). (**D**) Plot of the percentages of the products of the isomerization of 1,5-intrastrand CL of BBR3464 [adduct (%)] in the 20-bp duplexes 5′–5′ and 3′–3′ versus time: (open square), 1,3-interstrand CL formed in the 5′–5′ orientation in the duplex 5′–5′ (1,3-IEC 5′–5′); (open circle), 1,3-intrastrand CL formed in the bottom strand of the duplex 5′–5′ (1,3-IAC 5′–5′); (filled square), 1,3-interstrand CL formed in the 3′–3′ orientation in the duplex 3′–3′ (1,3-IEC 3′–3′); (filled circle), 1,3-intrastrand CL formed in the bottom strand of the duplex 3′–3′ (1,3-IAC 3′–3′). These percentages were calculated from the ratio of the radioactivity in each lane associated with the band corresponding to each product to the sum of the radioactivities associated with all bands (multiplied by 100). For other details, see the text.

migrating markedly more slowly at approximately the same rate as the duplex 5′–5′ containing single, site-specific 1,3-interstrand CL (Figure 3B, right). Moreover, another new band migrating slightly, but significantly faster than the band corresponding to the platinated top strand appeared at longer incubation times which comigrated with the unplatinated top single strand of the duplex 5′–5′. The analysis by FAAS and absorption spectrophotometry revealed no platinum contained in this product.

Similar experiments were also performed with the duplex 3′–3′ containing the single, site-specific 1,5-intrastrand CL of BBR3464. As a function of time, the radioactivity associated with the band corresponding to the bottom strand of this duplex 3′–3′ containing 1,5-intrastrand CL decreased [Figure 3A (left) and C], but markedly more slowly than in the case of the duplex 5′–5′ [Figure 3A (right) and C]. This new band migrated at the same rate as the duplex 3′–3′ (Figure 3A, left) containing single, site-specific 1,3-interstrand CL. This result demonstrates that similarly as in the case of the 1,5-intrastrand CL in the duplex 5′–5′ this intrastrand adduct formed in the duplex 3′–3′ was transformed into an interstrand CL as well, but much less readily. The platinated bases in the product resulting from the rearrangement of the intrastrand cross-linked duplex 3′–3′ were identified from Maxam–Gilbert (DMS) foot-printing as those involved in the interstrand CL formed between the 5′ G of the top strand and the 5′ G in the bottom strand of the duplex 3′–3′, i.e. in the 1,3-interstrand CL formed by BBR3464 in the 3′–3′ direction. The incubation of the duplex 3′–3′ containing the single, site-specific 1,5-intrastrand CL in which only the bottom strand was 5′-end labeled also resulted in a small amount of additional product when the platinated duplex 3′–3′ was incubated for a longer time (∼2 days) (Figure 3A, left). If the duplex 3′–3′ in which the top strand (containing 1,5-intrastrand CL of BBR3464) was only 5′-end-labeled with ^{32}P, the radioactivity associated with the band corresponding to the top strand of the duplex 3′–3′ containing 1,5-intrastrand CL decreased; concomitantly, the radioactivity associated with the new species migrating markedly more slowly at approximately the same rate as the duplex 3′–3′ containing single, site-specific 1,3-interstrand CL increased (Figure 3B, left). Moreover, another new band migrating slightly, but significantly faster than the band corresponding to the platinated top strand appeared at longer incubation times (∼2 days) which comigrated with the unplatinated top single strand of the duplex 3′–3′. The analysis by FAAS and absorption spectrophotometry revealed no platinum contained in this product. Thus, in contrast to the rearrangement of the 1,5-intrastrand CL formed by BBR3464 in the duplex 5′–5′, the rearrangement of this CL formed in the duplex 3′–3′ was relatively very slow.

The experiments described above were performed in the medium of 10 mM Tris–Cl (pH 7.4) and 150 mM NaClO₄, but identical yields of the rearrangement reactions were obtained if NaClO₄ was replaced by NaCl.

Differential scanning calorimetry

Microcalorimetric technique was used to characterize the effect of intrastrand and interstrand CLs of BBR3464 on the thermal stability and energetics of the 20-bp DNA duplexes 5′–5′ and 3′–3′ (for their sequences, see Figure 1C). Such thermodynamic data can show how the platinum CL influences duplex stability, a property that may affect stability and consequently susceptibility of these DNA lesions to isomerize. Recently, differential scanning calorimetry (DSC) was used to characterize the influence of different CLs of platinum antitumor drugs on the thermal stability and energetics of 15–20-bp DNA duplexes site-specifically modified by these drugs (23–25,28,29). We decided to expand these studies to 20-bp oligodeoxyribonucleotide duplexes containing unique CLs formed by BBR3464, namely 1,5-intrastrand CLs, and 1,3-intra and interstrand CLs which might occur as a consequence of the spontaneous rearrangement of the 1,5-intrastrand CL described above (Figure 4).

DSC melting profiles (ΔC_p versus T) of the parent un-modified duplexes 5′–5′ and 3′–3′ and the same duplexes containing a single, 1,5-intrastrand CL of BBR3464 in the top strand, 1,3-interstrand CLs formed in 5′–5′ or 3′–3′ direction, and 1,3-intrastrand CL in the bottom strand are shown in Figure 4A and B. Importantly, denaturation
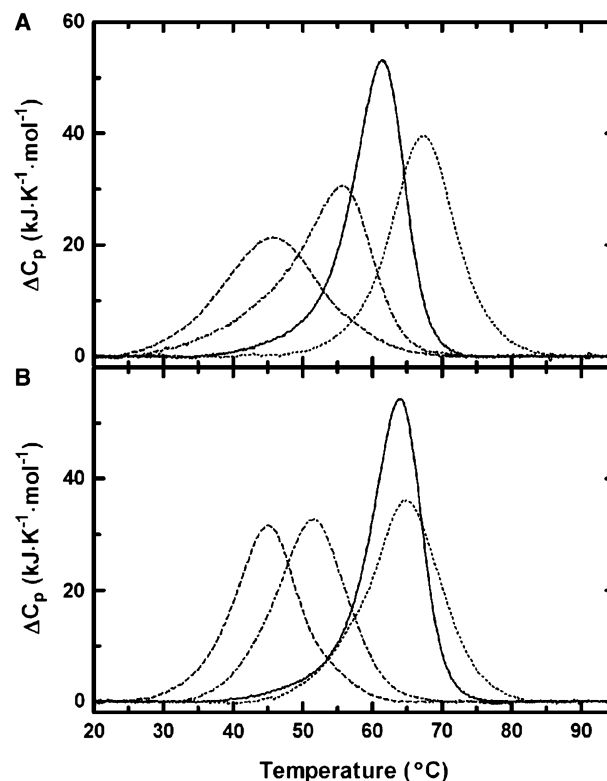

Figure 4. DSC thermograms for the duplexes 5′–5′ (**A**) and 3′–3′ (**B**) nonmodified (solid line) and containing a single 1,5-intrastrand CL in the top strand (dashed line), 1,3-interstrand CL (dotted line) or 1,3-intrastrand CL in the bottom strand (dot–dashed line) of BBR3464. The duplex concentration was 30 μM, and the buffer conditions were 10 mM phosphate buffer (pH 7) plus 150 mM NaCl. For other details, see the text.

(heating) and renaturation (cooling) DSC profiles of the unmodified or platinated duplexes were superimposable, which is consistent with the reversibility of the melting equilibrium; in addition, the first and subsequent DSC curves recorded for the same sample of the platinated duplexes were also superimposable (not shown). These results support the thesis that the adducts at elevated temperatures during the DSC experiment were thermally stable, i.e. that the elevated temperatures to which the platinated samples were exposed during the DSC experiment induced no adduct rearrangement, decomposition or dissociation of the platinum complex from the duplex. This thesis is also corroborated by the analysis of the platinated samples, which were exposed to the elevated temperatures during the DSC experiment, by platinum FAAS, gel electrophoresis under denaturing conditions and Maxam–Gilbert footprinting of platinum coordinated to G residues in DNA (26,30) (not shown). These analyses confirmed one molecule of BBR3464 bound to the platinated duplex and that the positions of the two platinated G residues in the duplexes containing single, site-specific intra- or interstrand CL remained unchanged during the DSC experiment.

All thermodynamic parameters discussed in this work (Table 1) refer to the duplex dissociation process. Differences in the dissociation thermodynamics due to the presence of an adduct are presented as '$\Delta\Delta$' parameters. These parameters are computed by subtracting the appropriate value measured for the control, the unplatinated duplex, from the value measured for the duplex containing the single, site-specific platinum adduct and are reported in Table 1 in parentheses. In addition, the transition entropy for a bimolecular complex depends on strand concentration. To eliminate the effect of different molecularities of the unplatinated or intrastrand and interstrand cross-linked duplex systems, we also performed a correction for this concentration dependence using the general procedure outlined

by Marky and Breslauer (31) to calculate a reduced (concentration independent) entropy (ΔS^*) (Table 1). More specifically, we subtracted out the portion of the entropy attributable to molecularity >1. For a bimolecular process this is $RT\ln(C_t/4)$ (where R is the gas constant and C_t equals to the total strand concentration). This term is derived from a model that assumes a truly two-state process, which is valid only for short oligonucleotides. The 20-bp duplexes used in this DSC study may be long enough to be approaching the pseudomonomolecular behavior of polymeric DNA. Therefore, entropy may be over-corrected by use of the standard model. Nevertheless, this should not compromise comparisons among $\Delta\Delta S$ and $\Delta\Delta G^0_{25}$ values within this study. On the other hand, this should be taken into consideration in the case when comparisons to corresponding $\Delta\Delta$'s from oligonucleotides of different lengths are made.

Inspection of the thermodynamic parameters in Table 1 reveals a number of interesting features. First, the formation of the CLs in the duplex 5′–5′ (1,5-intrastrand CL in the top strand, 1,3-interstrand CL in the 5′–5′ direction, or 1,3-intrastrand CL in the bottom strand) reduced the duplex 5′–5′ thermodynamic stability (ΔG^0_{25}), the trend was 1,5-intrastrand CL in the top strand >1,3-interstrand CL in the 5′–5′ direction >1,3-intrastrand CL in the bottom strand. In contrast, the formation of the CLs in the duplex 3′–3′ (1,5-intrastrand CL in the top strand, 1,3-interstrand CL in the 3′–3′ direction, or 1,3-intrastrand CL in the bottom strand) also reduced the duplex 3′–3′ thermodynamic stability (ΔG^0_{25}). The trend was similar as in the case of the duplex 5′–5′, but the difference between $\Delta\Delta G^0_{25}$ values obtained for 1,5-intrastrand and 1,3-interstrand CL in the duplex 3′–3′ was very small (cf. only 0.5 kJ mol^{-1} in the duplex 3′–3′ and 5.8 in the duplex 5′–5′).

Interestingly, the formation of all adducts of BBR3464 analyzed in the present work by DSC resulted in a large decrease in the enthalpy of duplex dissociation (Table 1). In other words, these adducts enthalpically destabilized the duplexes 5′–5′ and 3′–3′ relative to their unmodified counterparts. On the other hand, the formation of these adducts resulted in a substantial decrease in the duplex dissociation entropy (Table 1). Thus, the net result of these enthalpic and entropic effects was that the formation of the adducts byBBR3464 induced a decrease in the free energy of duplex dissociation at 25°C (ΔG^0_{25}; Table 1), this duplex destabilization being enthalpic in origin.

Table 1. Calorimetrically derived thermodynamic parameters for the dissociation (melting) of the 20-bp duplexes that are unmodified or contain a single, site-specific cross-link of BBR3464[a]

Duplex	ΔH_{cal}[b] (kJ mol^{-1})	ΔS (kJ K^{-1} mol^{-1})	ΔG^0_{25} (kJ mol^{-1})
5′–5′	−541.5	−1.535[c]	−83.7[c]
1,5-intrastrand CL	−397.2 (144.3)	−1.151[c] (0.384)	−54.0[c] (29.7)
1,3-interstrand CL	−480.4 (61.1)	−1.411 (0.124)	−59.8 (23.9)
1,3-intrastrand CL	−471.6 (69.9)	−1.356[c] (0.179)	−67.4[c] (16.3)
3′–3′	−546.1	−1.567[c]	−79.1[c]
1,5-intrastrand CL	−405.5 (140.6)	−1.192[c] (0.375)	−50.1[c] (29.0)
1,3-interstrand CL	−492.6 (53.5)	−1.482 (0.085)	−50.6 (28.5)
1,3-intrastrand CL	−426.0 (120.1)	−1.244[c] (0.323)	−55.2[c] (23.9)

[a]The '$\Delta\Delta$' parameters are given in parentheses (these parameters are computed by subtracting the appropriate value measured for the control, the unmodified duplex, from the value measured for the duplex containing the single, site-specific CL).
[b]Model-independent calorimetric enthalpies ΔH_{cal} were derived from DSC experiments.
[c]Denotes reduced values (for the details, see the text). Reduced free energy ΔG^0_{25}* (at 25°C) was calculated from the observed ΔH_{cal} values and the reduced entropy ΔS^* values.

CONCLUDING REMARKS

We discovered during the course of the studies of DNA modifications by antitumor trinuclear and bifunctional Pt[II] complex, BBR3464 that some CLs of this platinum compound were not stable. This observation prompted us to undertake a systematic study of the stability of the CLs formed in DNA by BBR3464. In this article, we show that under physiological conditions the coordination bonds between platinum and N7 of G residues involved in intrastrand or interstrand CLs of BBR3464 can be cleaved leading to the linkage isomerization reactions
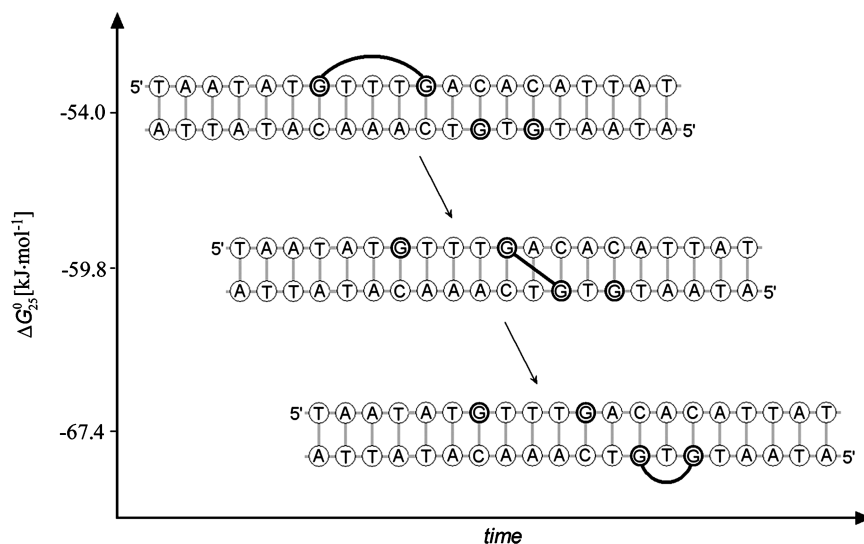
Figure 5. Scheme of the linkage isomerization reactions between BBR3464 and double-helical DNA substrate. The driving force that leads to the lability of DNA CLs of this metallodrug is a difference between the thermodynamic destabilization induced by the CL and by the adduct into which it isomerizes. Interestingly, the rearrangements proceed in the way that the CL originally formed in one strand of DNA can spontaneously translocate from one DNA strand to its complementary counterpart. ΔG^0_{25} values refer to the duplex dissociation process.

between this metallodrug and double-helical DNA substrate.

Upon incubation at 37°C of the 20-bp duplexes containing a single, site-specific 1,2-, 1,5- or 1,6-intrastrand CLs between G residues, we have shown the following main points: (i) the coordination bonds between Pt and the N7 of one of the G residues within the intrastrand adduct in the top strand are cleaved; (ii) the intrastrand CL cleavage leads to the formation of interstrand CLs, the rate of this linkage isomerization reaction is markedly higher if the interstrand CL is formed in the 5′–5′ orientation; (iii) the interstrand CLs react further to form intrastrand CL in the bottom strand; (iv) this successive rearrangement may proceed in the way that the molecule of BBR3464 originally coordinated to one strand of DNA can spontaneously translocate from this strand to its complementary counterpart via intermediate interstrand CL (Figure 5), which may evoke walking of this platinum complex on DNA molecule.

Migration of platinum complex from one strand to another in double-helical DNA has not been previously reported. This discovery reveals that reactions of platinum compounds with DNA can be unexpectedly complex, and has interesting implications about the importance of thermodynamic destabilization of DNA induced by the platinum lesions in determining target sites on DNA for binding of platinum drugs. In particular, the intrastrand and interstrand CLs of BBR3464 were found to be unstable if the sequence context enables the CL to rearrange into the new CL which thermodynamically destabilizes DNA considerably less. In other words, the results demonstrate that one of the driving forces that leads to the lability of DNA CLs of BBR3464 is a difference between the thermodynamic destabilization induced by the CL and by the adduct into which it could isomerize.

An understanding of how the physical and chemical differences of adducts of platinum antitumor compounds affect sequence selectivity of their DNA binding may provide insights into how to design more effective platinum agents or lead to the development of strategies to design new platinum agents targeting preselected nucleotide sequences in DNA forming in these sequences the most disruptive types of lesions. Furthermore, information of this type may provide a better understanding of the cytotoxic and mutagenic potential of specific lesions that are formed by platinum and perhaps also by DNA alkylating agents. There are some similarities in the mechanism of action of antitumor platinum complexes and DNA alkylating agents. Hence, interesting generalization of the results described in this work might be that migration of the DNA alkylating agents from one strand to another in double-helical DNA controlled by energetic signatures of these agents might be possible as well.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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