Molecular Interaction of DNA with Bisplatinum(II) Complexes Having Bis(Vicinal 1,2-Diamines) as Ligand†

Hildegard Büning,‡ Janina Altman,§ Wolfgang Beck,§ and Haralabos Zorbas*,‡

Institut für Biochemie der Ludwig-Maximilians-Universität München, Feodor-Lynen-Strasse 25, 81377 München, Germany, and Institut für Anorganische Chemie der Ludwig-Maximilians-Universität München, Meiserstrasse 1, 80333 München, Germany

Received March 31, 1997; Revised Manuscript Received July 7, 1997⊗

ABSTRACT: The interaction of novel, tetrafunctional bisplatinum compounds with DNA was investigated. These compounds have bis(vicinal 1,2-diamines) as ligand. The reactions' efficiency, types of cross-links, alterations of the global DNA structure, and sequence selectivity differ significantly from the corresponding features of cisplatin. In particular, they form multiple complexes with dsDNA, which include intrastrand, interstrand and interhelical cross-links and cross-links over sticky ends. The novel compounds are able to untwist but not shorten dsDNA. The reactivity and adduct-forming efficiency of these compounds is, depending on the spacer length, 100−200-fold higher than that of cisplatin. As a consequence, interstrand cross-links are also formed to a higher extent. The chemical stability of the interstrand cross-links against cyanide ions, however, is weaker than that of interstrand cross-links formed by cisplatin, suggesting that each platinum sphere of a bisplatinum compound forms intrastrand cross-links. With dsDNA, they show a preference toward purines, particularly guanines, but they apparently are also coordinated to other nucleobases. Their sequence selectivity toward dsDNA is higher than that of cisplatin. Thus, the novel compounds extend the spectrum of alternative platinum-based compounds with chemical features different from cisplatin.

Platinum complexes, like *cis*-diamminedichloroplatinum-(II) (*cis*-DDP,[1] cisplatin) and carboplatin, are administered in cancer diseases (Abrams & Murrer, 1993). In spite of good success in the treatment of some cancer forms, the application is compromised by severe side effects and natural or developing resistance. Several alternative platinum compounds have been synthesized with the ultimate goal to overcome the drawbacks of cisplatin (Abrams & Murrer, 1993). However, in order to address these shortcomings in a rational way, clinical studies must be supplemented with a detailed knowledge of the molecular action and cellular effects of platinum compounds. Cisplatin reacts with the cellular DNA, forming several covalent base modifications (Lepre & Lippard, 1990). Originally, it was thought that the base modifications simply cause inhibition of DNA replication and thereby cell death (Harder & Rosenberg, 1970; Howle & Gale, 1970). However, cell death does not necessarily correlate with inhibition of DNA replication (Sorenson & Eastman, 1988a). Instead, by formation of DNA adducts the drug seems to cause G2 arrest of the cell cycle and to trigger apoptosis (Barry et al., 1990; Chu, 1994; Sorenson et al., 1990; Sorenson & Eastman, 1988b). Although the signal transduction mechanism from DNA damage to the induction of programmed cell death remains unknown, disturbance of DNA secondary structure by specific types of platinum adducts seems to be among the capital causes for the lethal effects of cisplatin (Zamble & Lippard, 1995). Recently, the crystal (Takahara et al., 1995) and solution (Yang et al., 1995b) structure of the main adduct of cisplatin, d(GpG)-1,2 intrastrand cross-link, with dsDNA revealed that the DNA is untwisted and stably bent toward the major groove in a way that explains why it becomes a favored target for HMG-box proteins (Brown et al., 1993, Bruhn et al., 1992, 1993; Huang et al., 1994; Lawrence et al., 1993; McA'Nulty & Lippard, 1996; Pil & Lippard, 1992; Toney et al., 1989; Treiber et al., 1994; Zamble et al., 1996). The association of HMG-box proteins with this kind of lesions has been discussed as a cause of both the cytotoxicity and the developing resistance against cisplatin (Zamble & Lippard, 1995, and references therein). However, some cell lines like human Fanconi's anemia fibroblasts are sensitive against cisplatin, although they *can* repair 1,2-intramolecular cross-links. One reason for the sensitivity was suggested to be their incapability to repair d(GG)-intermolecular cross-links, which are minor DNA lesions (Dijt et al., 1988). The same is true for SUSA, a cell line from germ cell tumors of the testis, which is hypersensitive to cisplatin due to its inability to repair interstrand cross-links (Bedford et al., 1988; Hill et al., 1990). Elucidation of the structure of the d(GG)-interstrand cross-link provided a basis for the understanding of the differential cytotoxicity effects of intra- versus interstrand cross-links. In the past, the d(GG)-interstrand cross-link of cisplatin was repeatedly modeled in the major DNA groove (Brabec et al., 1993; Sip et al., 1992). However, recent NMR and migration studies in polyacrylamide gels revealed an entirely unexpected distortion of the

† This project was supported by a grant of the Deutsche Forschungsgemeinschaft to H.Z. and W.B. (Zo 59/4-1). This work is dedicated to Ms. Maria Stafilidou, 11741 Athens, Greece, who liked chemistry.

* To whom correspondence should be addressed. Tel: +49-89-74017-450. FAX: +49-89-74017-448. E-mail: zorbas@LMB.uni-muenchen.de.

‡ Institut für Biochemie.

§ Institut für Anorganische Chemie.

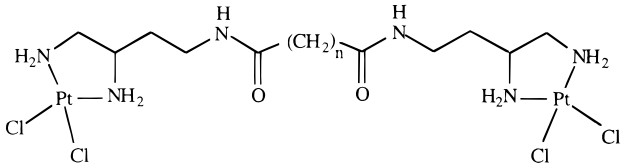
⊗ Abstract published in *Advance ACS Abstracts,* September 1, 1997.

[1] Abbreviations: bp, base pair(s); bPt, bisplatinum; DDP, diamminedichloroplatinum(II); ds, double strand; DMS, dimethyl sulfate; DMSO, dimethyl sulfoxide; HMG, high-mobility group; ss, single strand; T4 pol, T4 DNA polymerase.

S0006-2960(97)00741-1 CCC: $14.00

DNA structure which is bent toward the *minor* groove, locally unwound, and left-handed with extrahelical cytosines and with the drug lying in the *minor* groove (Huang et al., 1995; Paquet et al., 1996). Thus, although the interstrand adduct of cisplatin can also be recognized by HMG proteins *in vitro* (Kasparkova & Brabec, 1995), in the cell, different proteins may associate with this unique structure. Therefore distinct cellular detoxifying mechanisms may recognize and handle DNA adducts which effect different alterations in DNA structure.

At least two reasons are possible, due to which enhanced formation of interstrand cross-links might lead to better efficacy of platinum-based drugs. One reason could be that it might be more difficult or impossible for the cell to repair abundant interstrand cross-links or become resistant against them as opposed to intrastrand cross-links. A second, more "indirect" reason could be that rather unsuccessful attempts of the mismatch repair system activated by the specific DNA structure alterations due to interstrand cross-links may cause cell lethality by futile repair cycles. Whatever might be true, these suggestions inspired the recent development in synthesis of alternative platinum compounds which are supposed to show an enhanced formation of interstrand cross-links. These are the dinuclear bisplatinum complexes which display unique structural features in comparison with known cisplatin analogues (Farrell et al., 1990a; Roberts et al., 1989). Beck and co-workers have elaborated a general method of synthesis of dinuclear bis[platinum(II)] compounds $[Cl_2Pt(LL)PtCl_2]$ (Altman & Beck, 1995; Schuhmann et al., 1995). LL are two 1,2,4-triaminobutane units linked by nitrogen in position 4 as α,ω-dicarboxylic acid bis(amides) of variable chain length, $n = 4-6, 8$ [**1a** ($n = 4$), **1b** ($n = 5$), **1c** ($n = 6$), and **1d** ($n = 8$)] (see **1**). These complexes are potentially



1

tetrafunctional. Therefore, they may react differently with DNA, as compared to cisplatin. Here we show that these compounds share some features with cisplatin and other known dinuclear bis[platinum(II)] complexes (Farrell et al., 1990a; Roberts et al., 1989), but they also display several novel effects in the interaction with DNA.

MATERIALS AND METHODS

The synthesis of dinuclear bis[platinum(II)] compounds with the general formula $[Cl_2Pt(LL)PtCl_2]$ has been described (Altman & Beck, 1995; Schuhmann et al., 1995).

End-labeling of the monomeric double strand oligonucleotide was as described (Zorbas et al., 1989). For the construction of the dimeric oligonucleotide (see Figure 2), the top single strand was phosphorylated at the 5′-end with [γ-^{32}P]ATP and T4 polynucleotide kinase, annealed with the bottom single strand and the double-strand oligonucleotide was self-ligated with T4 ligase; this gave rise to only one sort of a dimeric double-strand molecule, with the *Hin*dIII sticky ends covalently ligated with each other.

cis$[Pt(NH_3)_2Cl_2]$ and each of the bPt complexes were prepared as a 4 mM stock solution in 100% dimethyl sulfoxide (DMSO) and stored at −20 °C. All DNA platination reactions were performed in aqueous solution (TE buffer at 10 mM Tris-HCl, pH 7.5, 1 mM EDTA) at a final DMSO concentration of 0.125−0.34%. The reactions were stopped by addition of 250 mM NaCl (final concentration).

For platination of linear DNA, 25 fmol (for simple titrations) or 75 fmol (for subsequent T4 pol footprinting analyzes) of dsDNA was incubated with increasing amounts of cisplatin (total range investigated: 50 nM−100 μM) or with one of the bPt complexes (total range investigated: 10 nM−5 μM) in TE, for 3 h at 37 °C in a final volume of 40 μL (final DMSO concentration: 0.125%). Nonreacted Pt complexes were removed by molecular sieve chromatography, and the DNA, or an aliquot thereof, was analyzed on a denaturing polyacrylamide gel.

For partial deplatination, dsDNA was first reacted with 100 μM cisplatin or 1 μM **1a**, as described above, in order to produce reasonable amounts of intra- and interstand cross-links. 100 fmol of platinated dsDNA was then subjected to treatment with 0.2 M NaCN, 20 mM Tris-HCl (pH 8.3), at 37 °C (Lemaire et al., 1991), in a total of 160 μL, and aliquots of 40 μL were removed after 20, 60, 120, and 240 min. The aliquots were purified from small molecules by molecular sieve chromatography, and the products of the partial deplatination were analyzed by denaturing polyacrylamide gel electrophoresis.

For detection of interhelical cross-links, 100 fmol of the 5′-end-labeled double-strand oligonucleotide, 350 fmol of the 3 kb plasmid, and increasing amounts of cisplatin (range: 50 nM−200 μM) or **1a** (range: 50 nM−60 μM) were incubated in TE for 3 h at 37 °C in a final volume of 30 μL (final DMSO concentration: 0.34%). The probes were divided into two parts. One part was analyzed in a native agarose gel (agarose concentration: 0.8%, with a lower part of 2%). The other part was analyzed in a denaturating polyacrylamide gel. In a pilot experiment (see Figure 3), dsDNA untwisting was examined by similar incubation of the 3 kb plasmid with 500 nM−60 μM **1a** without inclusion of the radioactive oligonucleotide and equivalent analysis in a native agarose gel (0.8%).

For preparative separation of **1a**−DNA complexes, 1.2 pmol of dsDNA was incubated with 500 nM **1a** in TE for 3 h at 37 °C in a final volume of 80 μL (final DMSO concentration: 0.125%). Nonreacted Pt complexes were removed by molecular sieve chromatography, and the DNA was separated on a denaturing polyacrylamide gel and autoradiographed. Complex areas I, II, and III were cut from the gel and eluted by diffusion in TE overnight at room temperature. The eluates were dialyzed against 0.1× TE and concentated in a Speedvac to give a final concentration of 1× TE. A final desalting step was performed by molecular sieve chromatography, after which the probes were subjected to T4 pol digestion, as described below.

For mapping of Pt lesions by T4 pol digestion, 75 fmol of the 5′-end-labeled dsDNA was incubated with increasing amounts of cisplatin (range: 50 nM−100 μM) or **1a** (range: 50 nM−1 μM), as described above. Nonreacted bPt complexes were removed by molecular sieve chromatography. Aliquots were analyzed in a denaturating polyacrylamide gel. The products of the binding reactions and unplatinated DNA, as control, were each digested with 12 units of T4 DNA polymerase in 50 mM NaCl, 10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM DTT, and 50 μg/mL

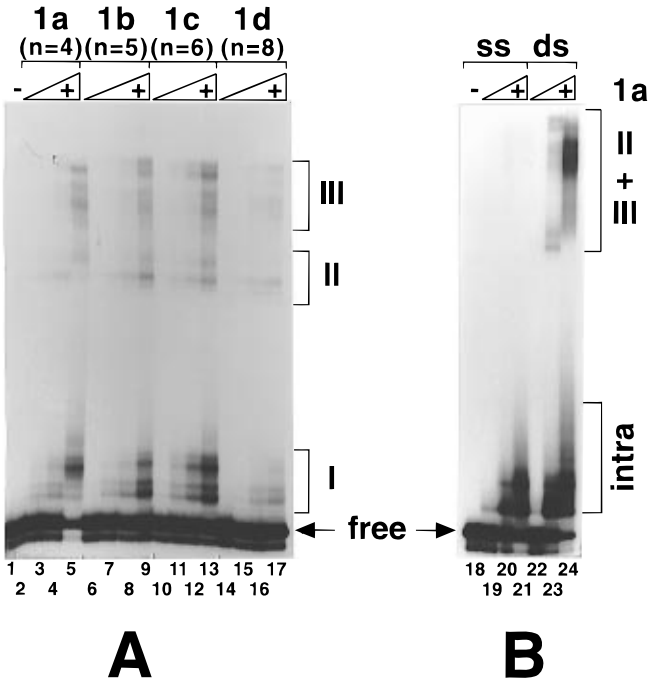


FIGURE 1: (A) Autoradiograph of the products of the interaction of bisplatinum compounds, as indicated (50 nM in lanes 2, 6, 10, and 14; 100 nM in lanes 3, 7, 11, and 15; 250 nM in lanes 4, 8, 12, and 16; 500 nM in lanes 5, 9, 13, and 17), with radioactively labeled dsDNA after electrophoretic separation on a denaturing polyacrylamide gel. Free DNA and distinct complex areas (I, II, and III) are indicated. (B) Same as in A, after interaction with **1a** with radioactively labeled ss- or dsDNA, as indicated (50 nM in lanes 19 and 22; 500 nM in lanes 20 and 23; 1000 nM in lanes 21 and 24).

BSA in a final volume of 50 μL for 30 min at 37 °C. Digested mononucleotides were seperated by molecular sieve chromatography. Pt adducts were removed by incubation in 250 mM NaCN (final concentration) overnight at 45 °C and separated from the DNA by molecular sieve chromatography. Digestion products were analyzed in a denaturating polyacrylamide gel.

Maxam and Gilbert sequencing reactions were performed as described (Zorbas et al., 1989). Calculations for estimating bPt dimensions were performed by HYPERCHEM and for estimating distances in dsDNA by INSIGHT programs.

RESULTS

Cross-Link Types of Bisplatinum Compounds and Global Effects on DNA Structure. In order to analyze the complexes of bPt with dsDNA, we labeled with ^{32}P the 5′-end of the top strand of a guanine-rich double-stranded oligonucleotide with the naturally occurring sequence

```
5'-AGC TTC AGA GGG GAC TTT CCG AGA GG-3'
     3'-AG TCT CCC CTG AAA GGC TCT CCA GCT-5'
```

from the enhancer of the light κ-chain gene and let it react with each one of the bPt compounds. The reaction products were separated electrophoretically in a denaturing polyacrylamide gel and analyzed autoradiographically (Figure 1A). The degree of the total modification of the DNA could be estimated from the densitometrically determined ratio of retarded DNA and total (retarded+free) DNA; in the experiments described here it was between 5% and 50%. From this analysis, we concluded that the bPt compounds form multiple complexes with DNA (complex areas I, II, and III; Figure 1A) and that the reactivity of the four compounds toward DNA differs. Although compound **1c** seemingly formed more complexes with DNA than the other three (Figure 1A, lane 13), the *ratio* of products to free DNA for **1a** at equivalent concentrations was much greater than for the other compounds (Figure 1A, lane 5). The reason for this apparent anomaly is that, at 500 nM, several **1a**−DNA products of a higher complexity were prevented from running into the gel matrix (not shown). Therefore, the order of reactivity of the compounds is **1a** ($n = 4$) > **1c** ($n = 6$) ≥ **1b** ($n = 5$) ≫ **1d** ($n = 8$). The different reactivity is apparently a consequence of the difference in the length of the spacer and points toward distinct stereospecific requirements for adduct forming with DNA.

In order to determine the nature of complexes of bPt with DNA, we compared complex formation of bPt compound **1a** with the double-strand oligonucleotide versus the top single strand of it, by the same methods. Reaction of **1a** with the single strand led to formation of only complex area I (Figure 1B), revealing that the latter consisted of intrastrand cross-links (and possibly monofunctional adducts), whereas complex areas II and III consisted of DNA molecules with at least one interstrand cross-link. However, since the ds-oligonucleotide we used possesses sticky ends, in principle two kinds of interstrand cross-links are possible: (a) between the two complementary strands of the same DNA molecule; (b) between two strands of two distinct dsDNA molecules which are transiently held together via their sticky ends. To distinguish between these two possibilities, we repeated the platination of the ds oligonucleotide, after its ends were made flush by filling them in with Klenow DNA polymerase (Zorbas et al., 1990). By this treatment, we expected the second case not to occur. In fact, only complex area III (and I) appeared in the result (Figure 2), consistent with the interpretation that complex area II was due to interstrand cross-links between two dsDNA molecules over sticky ends. At this stage, it was not known whether the individual bands within the different complex areas were due to distinct, to multiple successive, or to multiple disperse adducts on the DNA molecules (cf. below).

We next investigated the effect of platination on the global secondary structure of a circular ∼3 kb long dsDNA plasmid. For this purpose, increasing amounts of compound **1a** were incubated with the plasmid, and the products were analyzed in a neutral agarose gel (Figure 3). Platination of the plasmid effected a retardation of the negatively superhelical form of the plasmid (Figure 3, lanes 3−9) reflecting a global untwisting of the DNA.

To check for the capability of bPt to form interhelical (interduplex) cross-links, the same dsDNA plasmid and a radioactively labeled double-strand oligonucleotide were incubated with increasing amounts of **1a**, and the reaction products were analyzed electrophoretically in a neutral agarose gel. To visualize the plasmid, the gel was stained with ethidium bromide (Figure 4A). The location of the radioactive oligonucleotide was visualized by autoradiography (Figure 4B). As is seen in Figure 4B, above a certain concentration, radioactive material comigrates with plasmid bands. (Excess oligonucleotide is visible at the lower part of the gel, caught by an agarose of higher concentration.) This comigration can only be explained by association of the radioactive oligonucleotide with the circular plasmid

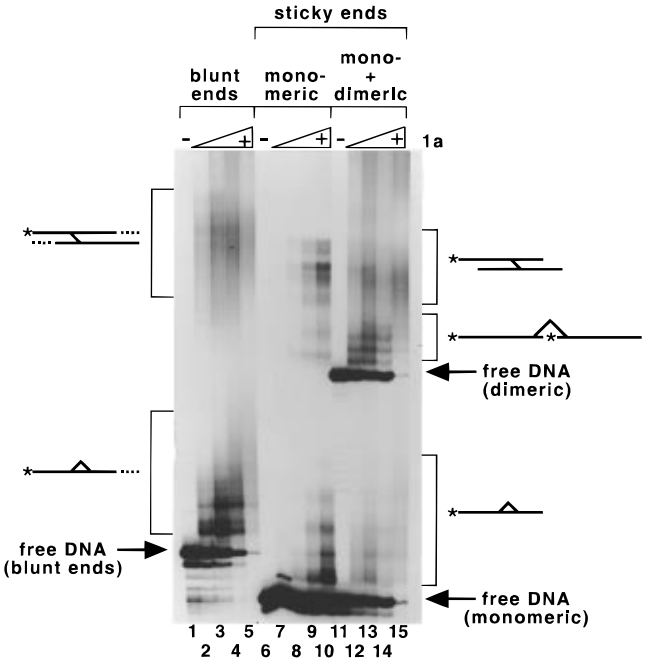


FIGURE 2: Autoradiograph of the products of the interaction of compound **1a**, as indicated (50 nM in lanes 2, 7, and 12; 100 nM in lanes 3, 8, and 13; 250 nM in lanes 4, 9, and 14; 500 nM in lanes 5, 10, and 15), with radioactively labeled monomeric (lanes 1−10), or a mixture of monomeric and dimeric (lanes 11−15), blunt-end (lanes 1−5), or sticky-end (lanes 6−15) dsDNA after electrophoretic separation on a denaturing polyacrylamide gel. Possible structures of the products are indicated on the margins.

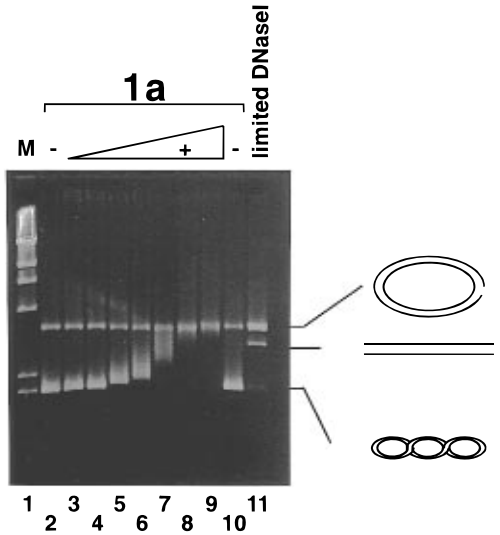


FIGURE 3: Ethidium bromide staining of the products of the interaction of compound **1a** (500 nM in lane 3; 1 μM in lane 4; 5 μM in lane 5; 10 μM in lane 6; 20 μM in lane 7; 40 μM in lane 8; 60 μM in lane 9) with plasmid DNA after electrophoretic separation on a native agarose gel. Lanes 2 and 10, no platinum addition. Lane 11, limited DNase I digestion of plasmid DNA. Nicked, linear, and superhelical plasmid forms are indicated on the right margin. M (lane 1), λ-dsDNA molecular weight marker cleaved with *Hin*dIII.

through interhelical cross-links via **1a**. We could show that at comparable and even higher concentrations, cisplatin did not give rise to interhelical cross-links whatsoever; all other complex types were formed, as checked by analysis in a denaturing polyacrylamide gel (data not shown). The inability of cisplatin to form interhelical cross-links is likely due to steric insufficiency as opposed to the longer bPt compounds.

In summary, compound **1a** is capable of forming intrastrand, interstrand and interhelical cross-links, cross-links over sticky ends, and of unwinding dsDNA. Similar results with only slight differences in efficiency were obtained with all other bPt compounds, **1b**, **1c**, and **1d** (data not shown).

Efficiency and Stability of Interstrand Cross-Links Formed by Bisplatinum as Compared to Cisplatin. One of the main purposes for the synthesis of the bPt compounds (Altman & Beck, 1995; Schuhmann et al., 1995) was to achieve a greater efficiency in forming interstrand cross-links with dsDNA than that of cisplatin. Formation of adducts of either **1a** or cisplatin with the radioactively labeled double-strand oligonucleotide at increasing concentrations of the two compounds was therefore compared with each other (Figure 5A). Under our experimental conditions (3 h of incubation), complexes in the interstrand cross-link area became evident at a concentration of 500 nM bPt (Figure 5A, lane 4). In contrast, ~50 μM cisplatin was needed for a comparable amount of such complexes (Figure 5A, lane 9). The latter were estimated densitometrically to be about 2−5% of the total platinum adducts (data not shown). This value corresponds to the portion of cross-links of cisplatin with dsDNA known to be interstrand cross-links (Brabec & Leng, 1993; Leng & Brabec, 1994). Therefore, these complexes most likely represented pure interstrand cross-links void of other complex types (e.g., intrastrand cross-links, and monofunctional adducts), as opposed to the "inter" complexes in lanes 5 and 10 in Figure 5A. Thus, from the comparison of lanes 4 and 9, we may conclude that, at *equivalent ligand concentrations*, **1a** may form interstrand cross-links roughly 100-fold more efficiently than cisplatin. However, inspection of the ratios of inter- to intrastrand cross-links at an *equivalent total degree of DNA modification* revealed that the portion of interstrand cross-links of **1a** appeared to be insignificantly larger than that of cisplatin (compare lane 4 vs lane 9 in Figure 5A). This suggests that bPt formed more interstrand cross-links because it was apparently more reactive than cisplatin and not due to an inherent bias toward inter- as opposed to intrastrand cross-links.

Complex formation of dsDNA with either **1a** at 500 nM or cisplatin at 50 μM was also monitored on a time scale from 15 min to 24 h to determine the reactions' equilibria. Incubation with **1a** reached a plateau yielding a pattern similar to that shown in Figure 5A, lane 4, within 6 h (data not shown). Incubation with cisplatin also reached a plateau after 6 h comparable to the complex pattern in Figure 5A, lane 9, which stayed the same also after prolonged incubation, in spite of the much higher initial concentration (data not shown). These results suggest that, besides the faster kinetics of the reaction, the higher efficiency of bPt as compared to cisplatin is also mirrored in a more favorable thermodynamic equilibrium toward the Pt−DNA complexes.

In order to compare the relative stability of inter- versus intrastrand cross-links, we treated DNA, which had been platinated either with **1a** or with cisplatin, with NaCN at nearly neutral pH for different times and analyzed the products on a denaturing polyacrylamide gel (Figure 5B). Several lesions could be reversed by this treatment. The reversal of intrastrand cross-links (and possibly monoadducts) of both compounds reached a plateau with no apparent further progression of deplatination of particular lesions (see Figure 5B, lower part of the autoradiograph). Deplatination of DNA treated with cisplatin affected the area of interstrand

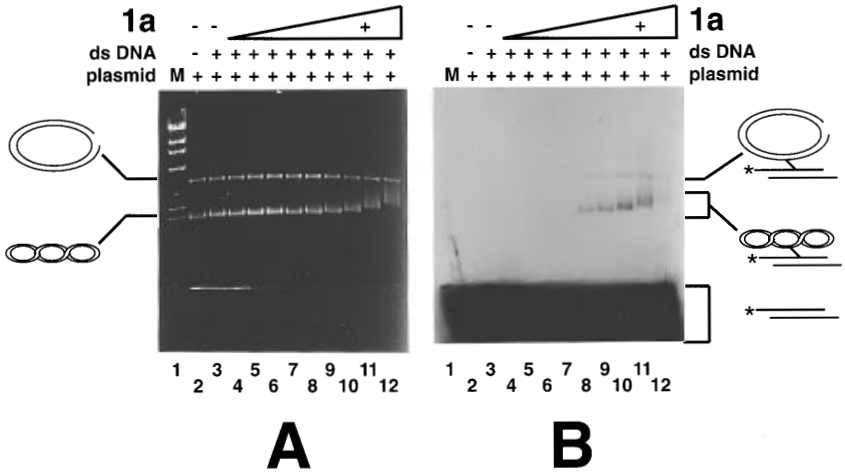


FIGURE 4: (A) Ethidium bromide staining of the products of the interaction of compound **1a** with circular plasmid DNA and radioactively labeled linear dsDNA, as indicated (50 nM in lane 4; 100 nM in lane 5; 500 nM in lane 6; 1 μM in lane 7; 5 μM in lane 8; 10 μM in lane 9; 20 μM in lane 10; 40 μM in lane 11; 60 μM in lane 12), after electrophoretic separation on a native agarose gel. Nicked and superhelical plasmid forms are indicated on the left margin. M (lane 1), λ-dsDNA molecular weight marker cleaved with *Hin*dIII. (B) Autoradiograph of the gel in A. Possible structures of the products are indicated on the right margin.

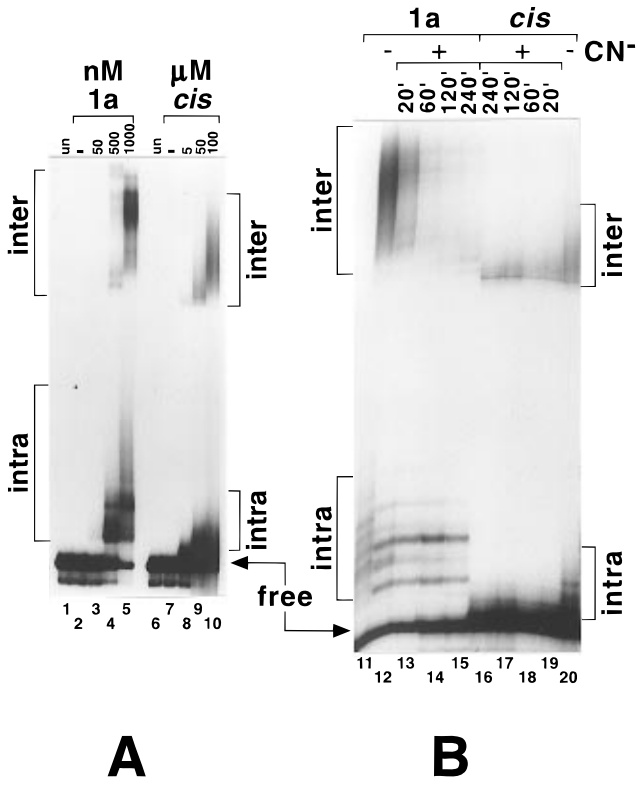


FIGURE 5: (A) Autoradiograph of the products of the interaction of **1a** (lanes 3−5) or cisplatin (*cis*, lanes 8−10) at the concentrations indicated ("−", lanes 2 and 7, no platinum compound included), with radioactively labeled dsDNA after electrophoretic separation on a denaturing polyacrylamide gel; "un" (lanes 1 and 6) refers to untreated DNA. Free DNA and complex areas with intrastrand (intra) and interstrand (inter) cross-links are indicated with brackets. (B) Autoradiograph of the deplatination kinetics of DNA platinated either with **1a** (1 μM, lane 11) or cisplatin (100 μM, lane 20) by cyanide ions (CN^-), for the times indicated (lanes 12−19). Free DNA and complex areas indicated as in panel A.

cross-links to a certain extent but left over a clear predominant interstrand cross-link representing about 50% of this area (Figure 5B, lanes 16−19, upper part of the autoradiograph). In contrast, cyanide ion treatment of DNA platinated with **1a** abolished virtually all of the interstrand cross-links (Figure 5B, lanes 12−15, upper part of the gel). Therefore it seems that interstrand cross-links formed by the bPt compounds are more labile against cyanide ions and hence of different quality than those formed by cisplatin.

In conclusion, the above experiments showed that the bPt compounds were more efficient than cisplatin in forming interstrand cross-links due to an overall higher rate and higher yield of Pt−DNA complexes. The chemical stability of the interstrand cross-links of bPt, however, was lower than that of cisplatin.

Sequence Preference of Bisplatinum Compounds. For determining the sequence preference of the bPt compounds we applied the T4 pol footprinting method. In the absence of nucleoside triphosphates, this polymerase possesses a 3′→5′ proofreading activity on single- or double-stranded DNA, but it is inhibited in its processivity by several DNA adducts one or two nucleotides in front of the lesion (Doetsch et al., 1985; Malinge et al., 1987; Panigrahi & Walker, 1990). For this type of analysis, the "top" strand of the same oligonucleotide as above was radioactively labeled, platinated with **1a** in either the single- or double-strand state (see Figure 6A), and digested exonucleolytically by T4 pol to completion, and the digestion products were analyzed in a denaturing gel (Figure 6B). Gel lane 7 in Figure 6B, representing the T4 pol stops on dsDNA, was analyzed quantitatively by a PhosphorImager (result in Figure 6C). The definition of a stop representing primary reaction sites was set arbitrarily at >150 cpm, i.e. above "weak" signals. According to this definition, T4 pol stops preferentially in front of particular purines giving "strong" signals at nucleotides A_9, G_{10}, G_{11}, A_{14}, A_{22}, and G_{25}, and "superstrong" signals at nucleotides G_{12} and G_{26} (arrows in Figure 6B and 6C).[2] Accepting that T4 pol stops one nucleotide in front of the lesion (Farrell et al., 1990a), this result suggests that bPt was coordinated preferentially at G_8, A_9, G_{10}, G_{11}, G_{13}, G_{21}, A_{24}, G_{25}, and/or G_{26} (for an overview, see Figure 9). The ambiguity of the two 3′-terminal coordination sites is due to the fact that coordination of bPt at the terminal G_{26} itself would lead to

[2] One must take into account that T4 pol cleavage of a 5′-labeled fragment up to nucleobase B_n yields a band which migrates ~1.5 bands slowler than the corresponding band B_{n-1} of the Maxam and Gilbert sequence reference; this last lacks the 3′-terminal nucleoside, which accounts for the one band difference, but carries a 3′-terminal phosphate, which effects the faster migration of 0.5 band (Tapper & Clayton, 1981).

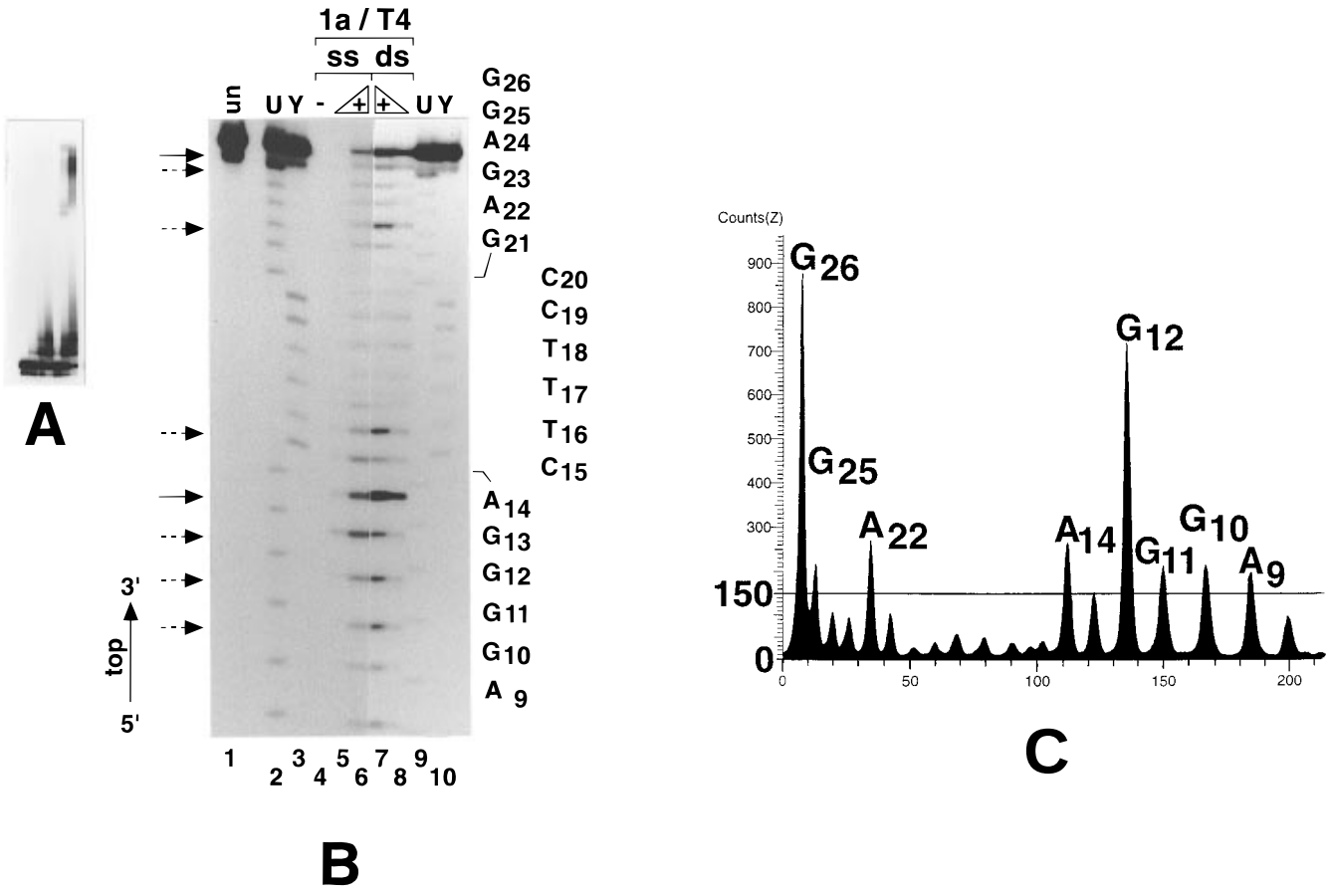


FIGURE 6: (A) Same as in Figure 1B. (B) Autoradiograph of the products of the interaction of different amounts of compound **1a** (500 nM in lanes 5 and 8; 1 μM in lanes 6 and 7) with radioactively 5′-labeled top strand of ss- (lanes 5 and 6), or dsDNA (lanes 7 and 8) after exonucleolytic digestion with T4 DNA polymerase (T4, lanes 4−8; "−", lane 4, no **1a** included in the reaction) and electrophoretic separation on a denaturing polyacrylamide gel; "un" (lane 1) refers to untreated DNA; U (lanes 2 and 9), purine sequencing ladders; Y (lanes 3 and 10), pyrimidine sequencing ladders. Coordinates of bases are indicated on the right margin. Solid and broken arrows on the left margin indicate "superstrong" and "strong" T4 pol stops, respectively, on the dsDNA (cf. text). (C) Radiation profile of the bands in lane 7 as quantified by PhosphorImager analysis (cf. text).

the same cleavage pattern as coordination at G_{25}. There is no way to distinguish between the possibilities of bPt coordination at G_{25} or G_{26} or both. The same is true for the terminal G_{25} and A_{26} of the bottom strand (see below). T4 pol also stops in front of all other purines with more or less equivalent frequency ("weak" signals), which, however, is much less than that of the primary reaction sites. T4 pol does not stop in front of pyrimidines, which suggests that bPt does not react with these bases. The nearly equal intensity of strong stops suggests that the coordinated bases reacted with nearly equal affinity with bPt.

Analysis of the platinated top *single* strand (Figure 6B, lanes 5 and 6) displayed the same stop signals as the double strand except those at A_{22} and G_{25}, suggesting that the bPt molecules coordinated at G_{21} and A_{24} in the double strand (see above) must have been involved *exclusively* in interstrand cross-links.

The assignment of intra- and interstrand cross-links in the double strand was pursued in more detail by an alternative experiment. For this purpose, the double-stranded oligonucleotide, radioactively labeled at the 5′-end of the top strand, was platinated with **1a** in a preparative scale and separated electrophoretically in a denaturing gel, and the products of complex areas I, II, and III were extracted from the gel (Figure 7A) and subjected to T4 pol digestion (Figure 7B). The complexes of area II could not be further processed due to limited material. The resulting stop pattern of area I displayed in Figure 7B, lane 8, confirmed the contacts at G_{21} and A_{24} as being exclusively interstrand cross-links, since they were entirely absent from this area. Furthermore, since the signal corresponding to contact G_{21} was also absent from area III (Figure 7B, lane 9), this bond may have only connected two strands over sticky ends with *no additional* interstrand cross-links. After calculations with the HYPERCHEM and INSIGHT programs, **1a** amounts to ∼24 Å in the extended conformation; therefore, all bonds over sticky ends resulting in coordinated positions ≤24 Å apart would be possible without requiring DNA distortion. Accordingly, bonds between the N^7 of G_{21} and, e.g., the O^4 of T_1 (21.5 Å) or the N^7 of G_3 (23.7 Å; guanosine in the *syn* conformation) of a second annealed oligonucleotide would be possible. However, this suggestion has to await further confirmation by the direct analysis of the complexes in area II. Stop signals with equivalent intensity in areas I and III (Figure 7B, lanes 8 and 9, respectively) corresponding to contacts in area G_8−G_{13} must have been part of intrastrand cross-links (or monofunctional adducts) which may or may not have also extended to interstrand bonds. Interestingly, the stop signal at G_{26} of area I appeared to be considerably weaker than in area III (compare lane 8 with lane 9 in Figure 7B), although the stop signals at G_{26} on the ssDNA and whole dsDNA were of comparable intensity (compare lane

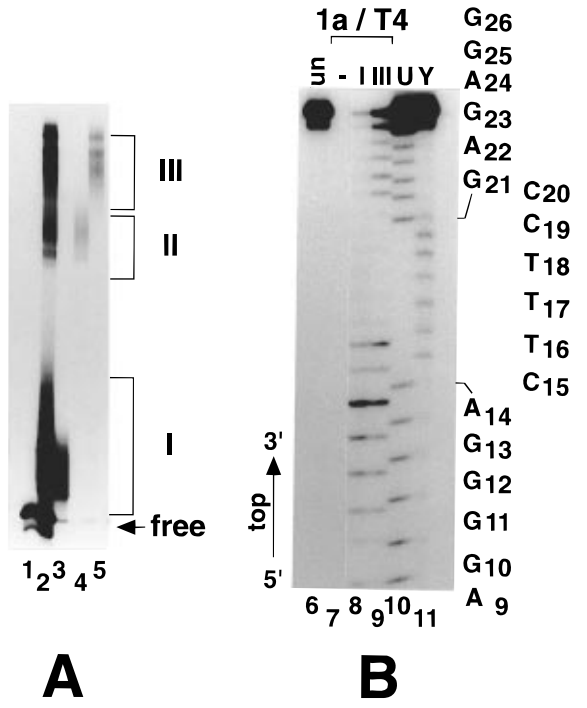


FIGURE 7: (A) Autoradiograph of aliquots of complex areas I (lane 3), II (lane 4), and III (lane 5) after electrophoretic separation and extraction of the products of the interaction of **1a** (500 nM) with radioactively labeled dsDNA (lane 2) from a denaturing polyacrylamide gel. The radiation intensities correspond to the extraction yields of the different complex types. The untreated DNA is shown in lane 1. (B) Autoradiograph of extracted complexes from area I (lane 3) and III (lane 5) of the interaction of **1a** with radioactively 5′-labeled top strand of dsDNA after exonucleolytic digestion with T4 DNA polymerase (T4, lanes 7−9, as indicated; "−", lane 7, no **1a** included in the reaction) and electrophoretic separation on a denaturing polyacrylamide gel; "un" (lane 6), untreated DNA; U (lane 10), purine sequencing ladder; Y (lane 11), pyrimidine sequencing ladder. Coordinates of bases are indicated on the right margin.

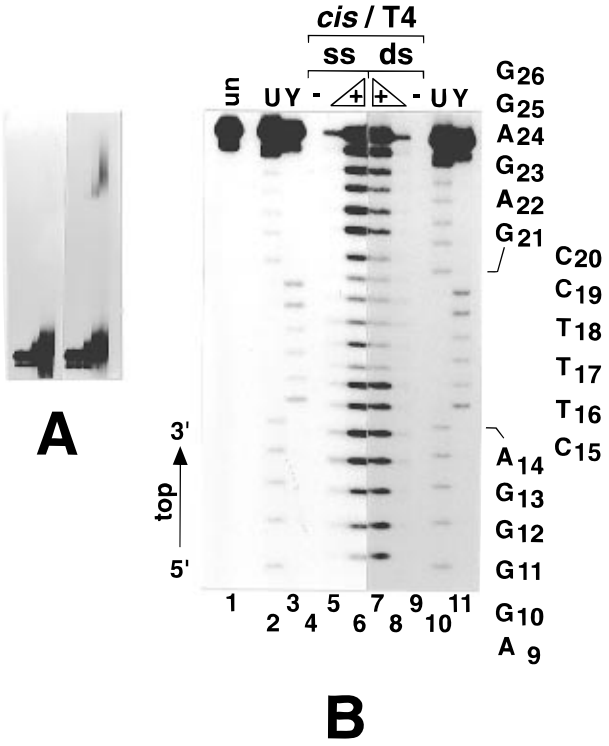


FIGURE 8: (A) Same as in Figure 6A; however, DNA is platinated with cisplatin at higher concentrations (5−100 μM; cf. text). (B) Autoradiograph of the products of the interaction of different amounts of cisplatin (5 μM in lanes 5 and 8; 50 μM in lanes 6 and 7) with radioactively 5′-labeled top strand of ss- (lanes 5 and 6) or dsDNA (lanes 7 and 8) after exonucleolytic digestion with T4 DNA polymerase (T4, lanes 4−9; "−", lanes 4 and 9, no cisplatin included in the reaction) and electrophoretic separation on a denaturing polyacrylamide gel; "un" (lane 1), untreated DNA; U (lanes 2 and 10), purine sequencing ladders; Y (lanes 3 and 11), pyrimidine sequencing ladders. Coordinates of bases are indicated on the right margin.

6 with lane 7 in Figure 6B). To explain this result, we propose that the contact(s) causing the stop at G_{26} (compare above) belonged to (an) *intrastrand* cross-link(s). On a portion of the *same* DNA strands carrying the intrastrand cross-link(s), however, additional **1a** molecule(s) must have been coordinated further upstream which formed interstrand cross-link(s) in dsDNA. Therefore, due to interstrand cross-link(s), this portion of the intrastrand cross-link(s) causing the stop at G_{26} was lacking from the separately analyzed area I (i.e., the fraction of platinated dsDNA void of interstrand cross-links; see Figure 7), because they must have been shifted to area III (see Figure 7). This phenomenon could not have been observed with platinated ssDNA versus platinated *whole* dsDNA (Figure 6B, lane 6 vs 7).

The coordination sites on the bottom strand in the double-strand oligonucleotide were also determined by the T4 pol method. In summary, we found that bPt was preferentially coordinated at C_{10}, T_{24}, G_{25}, and/or A_{26} (cf. above; data not shown). All results of the contact points of the ds oligonucleotide with **1a** are displayed in Figure 9.

The T4 pol experiments were repeated with a longer DNA fragment (114 bp) which encompasses the sequence of the oligonucleotide used. These experiments confirmed the preference of bPt for the same residues emphasized above (data not shown).

To determine possible differences of bPt from cisplatin in sequence preferences, the reaction sites of cisplatin with the double or top single strand of the same oligonucleotide were determined by the same T4 pol method (results of the top oligonucleotide in the double- and single-strand states are shown in Figure 8) and also quantitatively analyzed with a PhosphorImager (data not shown). Cisplatin displayed a general and rather even reactivity spectrum toward purines in the dsDNA substrate we used without the striking preference of bPt for certain residues, except for the two (or three; cf. above) 3′-terminal bases (Figure 8, lanes 7−9). This relaxed preference is most obvious in the 3′-region of the top strand (Figure 9), i.e., the region where interstrand bonds were exclusively formed with bPt (cf. above). Conclusively, bPt reacts more selectively with dsDNA than cisplatin. This was also reflected in experiments with the top single strand, in which also pyrimidines (mainly C_{15} and C_{19}) reacted with cisplatin to a certain extent (Figure 8, lanes 4−6). This last result with cisplatin was not unexpected, since the latter is known to be able to react with all nucleoside monophosphates in the order GMP > AMP ≫ CMP > T(U)MP (Lippert, 1989; Sherman & Lippard, 1987). Finally, since all contacts on the double strand (Figure 8, lane 7) were also present on the single strand (Figure 8, lane 6), no site on our DNA substrate seems to be suitable to exclusively form interstrand bonds with cisplatin, in contrast to bisplatinum. Thus, the distribution of intra- and interstrand

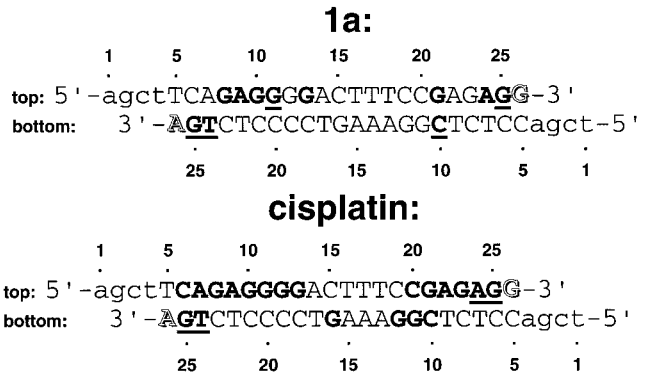


FIGURE 9: Summary of coordination sites of **1a** or cisplatin on dsDNA (cf. text). Boldface underlined uppercase letters, "super-strong" sites; boldface uppercase letters, "strong" sites; contour uppercase letters, ambiguous sites; lowercase letters, sites beyond gel resolution.

Table 1: Comparison of Reaction Features of Bisplatinum and Cisplatin with dsDNA

subject of analysis	novel bisPt compounds	cisplatin
reaction kinetics	fast	slow
reaction yield	high	low
intrastrand cross-links	+++++++++	+++
interstrand cross-links (ICLs)	+++	+
susceptibility of ICLs to cyanide	+	−
cross-links over sticky ends	+	ND
interhelical cross-links	+	−
untwisting of dsDNA	+	+
shortening of dsDNA	−	+
sequence selectivity	high	low

cross-links on dsDNA may also be different between bPt and cisplatin.

DISCUSSION

Cisplatin adducts on DNA effect definite structural distortions, like untwisting and bending (Huang et al., 1995; Takahara et al., 1995; Yang et al., 1995b). These structure alterations are considered to be the basis for cytotoxicity, as well as resistance of cells against cisplatin (Zamble & Lippard, 1995). Consequently, for alternative platinum compounds with the ultimate goal of stronger cytotoxicity, antitumor activity and/or suppression of emerging resistance, it should be required that they differ in their modes of reaction with DNA compared to those of cisplatin. With the experiments presented in this study, we found that the bPt compounds of the bis(amide) type fulfill this requirement since they display several differences to cisplatin. Important results from the analyzes of this study are summarized and compared with cisplatin in Table 1. It is evident that our bPt compounds display novel features in the interaction with DNA. Although none of the sites coordinated by bPt is not also coordinated by cisplatin (see Figure 9), the reaction's efficiency, types of cross-links, alterations of the global DNA structure, and site selectivity differ significantly. The stronger reactivity of the novel compounds with DNA reflected in faster kinetics and higher yield is probably due to the tetrafunctionality of bPt (higher local concentration of coordination sites) as well as to the higher flexibility and more suitable geometry of bPt.

For concentrated stock solutions, bPt compounds were first dissolved in DMSO at ambient temperature (see Materials and Methods). To maintain equivalent conditions, cisplatin was also first dissolved in DMSO. Solubilization of *cis*- or *trans*-DDP in DMSO at elevated temperatures effects the formation of derivatives (Kerrison & Sadler, 1977; Sundquist et al., 1987), of which the *trans*-$[Pt(NH_3)_2(DMSO)Cl]^+$ derivative displayed accelerated rates of DNA binding by a factor of 2−3 as compared to the parent compound *trans*-DDP (Sundquist et al., 1987). We have recently shown (Schuhmann et al., 1995) that our bPt complexes also undergo DMSO solvolysis (complete after 12 h at ambient temperature) by displacement of one chloride at each platinum by Pt−S-coordinated molecule of DMSO. DMSO derivatives may, in principle, also be partially responsible for the fast bPt kinetics of DNA binding (cf. *trans*-DDP paradigm, above). However, we consider it an unlikely reason for the pronounced *differences* in DNA binding kinetics between bPt and cisplatin. Firstly, after initial dissolving in DMSO at room temperature, bPt compounds and cisplatin were immediately used and/or immediately frozen at −20 °C. Analysis of interaction with DNA in aqueous solution was performed after dilution to reach a final DMSO concentration of 0.125−0.34%. Therefore, DMSO (and other) derivatives most probably could not form at all or formed to only a negligible extent. Secondly, if significant amounts of DMSO derivatives could form, bPt compounds may have been a mixture of all-cloride or chloride−DMSO on the side of the leaving groups with no changes occurring in amine group coordination and the five-membered chelate rings, as proved by NMR studies (Schuhmann et al., 1995). Cisplatin in DMSO gives rise primarily to *cis*-$[Pt(NH_3)_2(DMSO)Cl]^+$ (Kerrison & Sadler, 1977; Sundquist et al., 1987). Thus the configuration of our bPt compounds in DMSO with regard to the leaving groups might have been comparable to that of cisplatin in DMSO and, hence, most probably not the reason for the differences in the reactivity.

As a consequence of the overall stronger reactivity of bPt, the final amount of interstrand cross-links of all reacted DNA, at comparable cisplatin and bPt concentrations, was also higher. Roberts et al. (1989) reported that different tetrafunctional bPt compounds form a larger *portion* of interstrand cross-links with dsDNA than cisplatin, on the basis of the ratio of bound platinum per nucleotide. However, formation of interstrand cross-links by cisplatin may have been underestimated by a factor of 50 or more, as discussed in the same report. On the other hand, we cannot exclude that our tetrafunctional compounds might also be able to form a larger portion of interstrand cross-links with a different target DNA.

It has been reported that by mild cyanide ion treatment, it is possible to remove differentially cisplatin adducts which are not interstrand cross-links (Lemaire et al., 1991). By this method, we were able to show that, in fact, deplatination products of cisplatin converged mostly to a single persistent interstrand cross-link. Some intrastrand cross-links also survived after a 4-h treatment, which is in accord with the findings of other authors who reported that cyanide may not fully reverse intrastrand adducts of cisplatin (Lemaire et al., 1991; Malinge et al., 1987).[3] To our surprise, cyanide treatment almost entirely reversed the *interstrand* cross-links formed by bPt. This signifies that interstrand cross-links due to bPt may be of a different chemical quality than interstrand cross-links due to cisplatin. One possibility might be a structure in which each coordinated DNA site is bound to

another, not to the same platinum sphere of one bPt molecule. Thus, formally, this structure would resemble two intrastrand cross-links (or monoadducts, see below), which in turn might be susceptible to cyanide reversal. Alternatively, but not mutually exclusively, the different reactivity of the interstrand cross-links of bisplaninum vs cisplatin toward CN^- might also be due to different induced conformational changes of the DNA, not only to the different geometry of the drug's coordination.

The bPt compounds investigated here untwist, but do not shorten dsDNA. By the experiments performed in this study, we cannot exclude that locally our compounds may also induce DNA strand separation (unwinding). With respect to global untwisting, our compounds behave like cisplatin for which a similar phenomenon on the DNA secondary structure was reported earlier (Cohen et al., 1979). At the same time, however, they differ from cisplatin, since we did not observe an acceleration of the nicked plasmid form which would indicate DNA shortening (see Figure 3). In this regard, our *tetrafunctional* bPt compounds behave like dinuclear *bifuctional* bPt compounds, described recently (Farrell et al., 1995). Other dinuclear, tetrafunctional bPt compounds (Farrell et al., 1988) were first reported not to untwist DNA (Roberts et al., 1989). However, later studies which controlled the factual platination degree revealed that the opposite was the case (Keck & Lippard, 1992). Thus, DNA untwisting, or possibly unwinding, seems to be a phenomenon shared by all known dinuclear bPt compounds (Farrell et al., 1988, 1990a, 1995; Keck & Lippard, 1992; Roberts et al., 1989). Moreover, to our knowledge, no other bPt compound was reported to shorten or bend dsDNA either, which are characteristic consequences of bifunctional cisplatin adducts. The alterations of DNA structure due to bPt binding rather resemble the effects of *monofunctional* platinum binding brought about by mononuclear platinum compounds, e.g., chlorodiethylenetriamineplatinum(II) chloride {[Pt(dien)Cl]Cl} or *cis*-diamminemonoaquamonochloroplatinum(II) *cis*-$[Pt(NH_3)_2(H_2O)Cl]^+$ which unwind locally but do not bend dsDNA [Brabec et al., 1992, and references therein, 1994). An analogous monofunctional binding of each single (potentially bifunctional) platinum sphere of bPt to one coordination site would be in agreement with the hypothesis proposed above for the interstrand cross-links of bPt.

Interhelical cross-links first occurred above a **1a** concentration of ~5 μM (lane 8 in Figure 4B), an oligonucleotide concentration of 3.3 nM and a 3 kb plasmid concentration of 11.7 nM, which together correspond to a total DNA concentration of 26 bp length of ~1350 nM. These values are 10 times the **1a** and 2250 times the dsDNA concentration at which interstrand cross-links first became evident (e.g., Figure 5A, lane 4, with 500 nM **1a** and 0.6 nM DNA).[4] We conclude therefore, that complex area III (and II) as shown in Figure 1 most probably did not contain any oligonucleotide molecules that had been connected via interhelical cross-links under the experimental conditions applied. The considerably lower educt concentrations were evidently also the reason for the absence of interstrand cross-links between (noncomplementary) single strands, which formally correspond to interhelical cross-links (cf. Figure 1B, lanes 19−21, with 50−1000 nM **1a** and 0.6 nM DNA). The association of the radioactively labeled oligonucleotide with the circular plasmid was evidently stronger with the superhelical than with the nicked plasmid form (Figure 4B) in spite of the nearly equimolar amounts of both forms (Figure 4A). This might be due to a higher platination degree of the superhelical form. A higher affinity of bPt to the latter is probable, since the resulting untwisting effect, i.e., removal of superhelical turns (cf. above), is accompanied by a negative free-energy change of the system toward a less ordered, relaxed molecule [cf. Bauer and Vinograd (1970)].

For mapping the platinum lesions, we used T4 pol. It must be clearly said that one has to accept that T4 pol stops one nucleotide in front of the adduct. To our knowledge, there are no systematic studies on this assumption and at least one result is not in agreement with this statement. It has been shown on well-defined oligonucleotides modified by *trans*-DDP that T4 pol *stops one nucleotide before* the 3′-guanosine in the case of d(GTG)-1,3 intrastrand cross-link but *stops at the* 3′-guanosine in the case of d(GTTG)-1,4 intrastrand cross-link (Boudvillain et al., 1995). Bearing that in mind, with respect to the sequence preference, we found that our compounds clearly favor purines, particularly guanines. This might have been expected from the platinum−nucleobase chemistry (Lippert, 1989; Sherman & Lippard, 1987). However, there were also coordination sites to a cytosine and to a thymine (Figure 9; C_{10} and T_{24} on the bottom strand). Both of these residues were also coordinated by cisplatin (Figure 9). To our knowledge, cisplatin coordination to such bases in dsDNA has never been reported yet. The coordinated cytosine-10 (underlined) was part of the sequence 5′-CGGA-3′. Interestingly, cytosine coordination by both **1a** and cisplatin was also found at two additional sites having the sequence 5′-CGGA-3′ of a longer dsDNA fragment (114 bp) encompassing the oligonucleotide sequence (data not shown). Therefore, we believe that these particular sites are real and not artifacts of the T4 exonucleolytic method. Moreover, we would like to point out that other sequences (e.g., TACT·AGTA) which are not recognized targets of cisplatin have also been detected recently (Grimaldi et al., 1994).

C_{10} of the bottom strand, which is the only one residue in this area having firmly bound **1a** (see Figure 9), lies directly opposite the platinated G_{21} of the top strand, which was identified as an interstrand cross-link. This arrangement suggests a direct connection of these two residues. In principle, a d(GC) interstrand platinum cross-link in dsDNA is possible, as demonstrated with *trans*-platin (Brabec & Leng, 1993). However, cisplatin only forms d(GG) interstrand cross-links (Lemaire et al., 1991). Therefore, we consider it improbable that *one and the same* cisplatin moiety

[3] Persistent adducts of cisplatin or of **1a** were not the consequence of exhaustion of cyanide ions. 100 fmol of ds oligonucleotide was deplatinated in a total of 160 μL for the four time intervals shown in Figure 5B. Since the average degree of initial platination was ~50%, we may assume maximal platinum coordination at the half of all residues. Therefore, the initial amount of platinated mononucleotides was ~16 nM which is exceeded by the molarity of cyanide ions by a factor of $\sim 12.5 \times 10^6$.

[4] Analyzed amounts (at equivalent exposure times of the autoradiographs) of the two different experiments were different. Only ~2 fmol of DNA was analyzed in the denaturing polyacrylamide gels showing intermolecular cross-links, whereas each lane of the native gel shown in Figure 4 contained 50 fmol of oligonucleotide and 175 fmol of plasmid. This augments the difference in efficiency of forming the two types of cross-links even further.

of **1a** formed this putative interstrand cross-link. Thus, although the G_{21} contact of **1a** on the top strand has tentatively been assigned to a cross-link over sticky ends (see above), it is also possible that a d(GC) interstrand cross-link between the two complementary strands formed at this site in which each nucleotide was bound by a *different* coordination sphere of bPt. This arrangement would also be in accord with the proposed structure of interstrand cross-links formed by bPt inferred above. Validation of this point, however, requires further analysis.

Besides higher reactivity and adduct yield, the reasons for other differences between bPt and cisplatin, i.e. stability of interstrand cross-links, global effects on DNA structure, and sequence selectivity, all pointing toward a different structural association with DNA, are not immediately evident. However, we suggest that the exact structure of a cisplatin moiety may be significant for the reaction's outcome. For example, the presence of ring structures, as in our compounds, may influence significantly interaction with DNA. For comparison, replacement of NH_3 in some *cis*-, *trans*-, (Zou et al., 1993), and bisplatinum (Farrell et al., 1995) analogues by pyridine also causes profound changes in their interaction with DNA compared to that of the parental compounds, possibly due to a larger surface or by intercalation. In fact, NMR spectroscopy of a different bPt compound with a palindromic dsDNA fragment revealed a remarkable association with and unexpected alterations of the DNA structure (Yang et al., 1995a), e.g., location of the ligand and the coordinated guanines of the two opposite strands in the structure's minor groove and stabilization of an intramolecularly paired DNA. It is possible that bisplatinum complexes establish a new class of platinum-based compounds with unprecedented adducts thus causing unique effects on DNA structure.

What are the benefits we may expect from a possible clinical application of bPt compounds? Investigations with other dinuclear bPt compounds having a similar (Farrell, 1993; Farrell et al., 1990a,b; Kraker et al., 1992) as well as a different basic structure (Broomhead et al., 1993) have already demonstrated that most of them display a higher cytotoxicity and antitumor activity than cisplatin, at the same time being not cross-resistant to cisplatin. These characteristics may well be due to the enhanced formation of unique interstrand cross-links. A possible general feature of the bPt interstrand cross-links being the result of monofunctional coordination of the two platinum spheres as discussed above does not contradict the antitumor activity, which in fact may also be achieved by platinum agents having only one labile ligand (Hollis et al., 1991). Although experiments aimed at the biological activity of bPt compounds of the bis(amide) type are still in progress, we may assume quite similar biological effects, since they share similar chemical features and effects on DNA structure with the other bPt compounds (see above). Moreover, due to the overall higher reactivity, lower doses in medical application bypassing undesirable side effects of toxicity may be also considered.

ACKNOWLEDGMENT

We thank S. Werner and S. Wild for excellent technical assistance. We express our gratefulness to Dr. B. Steipe and B. Miller for computer modeling and calculations. We acknowledge the stimulation and continuous support of Prof. Dr. E.-L. Winnacker.

REFERENCES

Abrams, M. J., & Murrer, B. A. (1993) *Science 261*, 725−730.
Altman, J., & Beck, W. (1995) *Tetrahedron 51*, 13309−13320.
Barry, M. A., Behnke, C. A., & Eastman, A. (1990) *Biochem. Pharmacol. 40*, 2353−2362.
Bauer, W., & Vinograd, J. (1970) *J. Mol. Biol. 47*, 419−435.
Bedford, P., Fichtinger, S. A., Shellard, S. A., Walker, M. C., Masters, J. R., & Hill, B. T. (1988) *Cancer Res. 48*, 3019−3024.
Boudvillain, M., Dalbiès, R., Aussourd, C., & Leng, M. (1995) *Nucleic Acids Res. 23*, 2381−2388.
Brabec, V., & Leng, M. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 5345−5349.
Brabec, V., Reedijk, J., & Leng, M. (1992) *Biochemistry 31*, 12397−12402.
Brabec, V., Sip, M., & Leng, M. (1993) *Biochemistry 32*, 11676−11681.
Brabec, V., Boudny, V., & Balcarova, Z. (1994) *Biochemistry 33*, 1316−1322.
Broomhead, J. A., Rendina, L. M., & Webster, L. K. (1993) *J. Inorg. Chem. 49*, 221−234.
Brown, S. J., Kellett, P. J., & Lippard, S. J. (1993) *Science 261*, 603−605.
Bruhn, S. L., Pil, P. M., Essigmann, J. M., Housman, D. E., & Lippard, S. J. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 2307−2311.
Bruhn, S. L., Housman, D. E., & Lippard, S. J. (1993) *Nucleic Acids Res. 21*, 1643−1646.
Chu, G. (1994) *J. Biol. Chem. 269*, 787−790.
Cohen, G. L., Bauer, W. R., Barton, J. K., & Lippard, S. J. (1979) *Science 203*, 1014−1016.
Dijt, F. J., Fichtinger, S. A., Berends, F., & Reedijk, J. (1988) *Cancer Res. 48*, 6058−6062.
Doetsch, P. W., Chan, G. L., & Haseltine, W. A. (1985) *Nucleic Acids Res. 13*, 3285−3304.
Farrell, N. (1993) *Cancer Invest. 11*, 578−589.
Farrell, N., Qu, Y., Feng, L., & van Houten, B. (1990a) *Biochemistry 29*, 9522−9531.
Farrell, N., Qu, Y., & Hacker, M. P. (1990b) *J. Med. Chem. 33*, 2179−2184.
Farrell, N., Appleton, T. G., Qu, Y., Roberts, D. J., Soares Fontes, A. P., Skov, K. A., Wu, P., & Zou, Y. (1995) *Biochemistry 34*, 15480−15486.
Farrell, N. P., de Almeida, S. G., & Skov, K. A. (1988) *J. Am. Chem. Soc. 110*, 5018−5019.
Grimaldi, K. A., McAdam, S. R., Souhami, R. L., & Hartley, J. A. (1994) *Nucleic Acids Res. 22*, 2311−2317.
Harder, H. C., & Rosenberg, B. (1970) *Int. J. Cancer 6*, 207−216.
Hill, B. T., Shellard, S. A., Hosking, L. K., Fichtinger, S. A., & Bedford, P. (1990) *Int. J. Radiat. Oncol. Biol. Phys. 19*, 75−83.
Hollis, L. S., Sundquist, W. I., Burstyn, J. N., Heiger, B. W., Bellon, S. F., Ahmed, K. J., Amundsen, A. R., Stern, E. W., & Lippard, S. J. (1991) *Cancer Res. 51*, 1866−1875.
Howle, J. A., & Gale, G. R. (1970) *Biochem. Pharmacol. 19*, 2757−2762.
Huang, H., Zhu, L., Reid, B. R., Drobny, G. P., & Hopkins, P. B. (1995) *Science 270*, 1842−1845.
Huang, J. C., Zamble, D. B., Reardon, J. T., Lippard, S. J., & Sancar, A. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 10394−10398.
Kasparkova, J., & Brabec, V. (1995) *Biochemistry 34*, 12379−12387.
Keck, M. V., & Lippard, S. J. (1992) *J. Am. Chem. Soc. 114*, 3386−3390.
Kerrison, S. J. S., & Sadler, P. J. (1977) *J. Chem. Soc., Chem. Commun.*, 861−863.
Kraker, A. J., Hoeschele, J. D., Elliott, W. L., Showalter, H. D., Sercel, A. D., & Farrell, N. P. (1992) *J. Med. Chem. 35*, 4526−4532.
Lawrence, D. L., Engelsberg, B. N., Farid, R. S., Hughes, E. N., & Billings, P. C. (1993) *J. Biol. Chem. 268*, 23940−5.

Lemaire, M.-A., Schwartz, A., Rahmouni, A. R., & Leng, M. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 1982−1985.
Leng, M., & Brabec, V. (1994) *IARC Sci. Publ.*, 339−348.
Lippert, B. (1989) in *Progress in Inorganic Chemistry* (Lippard, S. J., Ed.) pp 1−97, J. Wiley & Sons, Inc., New York.
Lepre, C. A., & Lippard, S. J. (1990) in *Nucleic Acids and Molecular Biology* (Eckstein, F., & Lilley, D. M. J., Eds.) pp 9−38, Springer Verlag, Berlin, Heidelberg.
Malinge, J.-M., Schwartz, A., & Leng, M. (1987) *Nucleic Acids Res. 15*, 1779−1785.
McA'Nulty, M. M., & Lippard, S. J. (1996) *Mutat. Res. 362*, 75−86.
Panigrahi, G. B., & Walker, I. G. (1990) *Biochemistry 29*, 2122−2126.
Paquet, F., Pérez, C., Leng, M., Lancelot, G., & Malinge, J.-M. (1996) *J. Biomol. Struct. Dyn. 14*, 67−77.
Pil, P. M., & Lippard, S. J. (1992) *Science 256*, 234−237.
Roberts, J. D., van Houten, B., Qu, Y., & Farrell, N. P. (1989) *Nucleic Acids Res. 17*, 9719−9733.
Schuhmann, E., Altman, J., Karaghiosoff, K., & Beck, W. (1995) *Inorg. Chem. 34*, 2316−2322.
Sherman, S. E., & Lippard, S. J. (1987) *Chem. Rev. 87*, 1153−1181.
Sip, M., Schwartz, A., Vovelle, F., Ptak, M., & Leng, M. (1992) *Biochemistry 31*, 2508−2513.
Sorenson, C. M., & Eastman, A. (1988a) *Cancer Res. 48*, 6703−6707.
Sorenson, C. M., & Eastman, A. (1988b) *Cancer Res. 48*, 4484−4488.
Sorenson, C. M., Barry, M. A., & Eastman, A. (1990) *J. Natl. Cancer Inst. 82*, 749−755.
Sundquist, W. I., Ahmed, K. J., Hollis, L. S., & Lippard, S. J. (1987) *Inorg. Chem. 26*, 1524−1528.
Takahara, P. M., Rosenzweig, A. C., Frederick, C. A., & Lippard, S. J. (1995) *Nature 377*, 649−652.
Tapper, D. P., & Clayton, D. A. (1981) *Nucleic Acids Res. 9*, 6787−6794.
Toney, J. H., Donahue, B. A., Kellett, P. J., Bruhn, S. L., Essigmann, J. M., & Lippard, S. J. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 8328−8332.
Treiber, D. K., Zhai, X., Jantzen, H. M., & Essigmann, J. M. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 5672−6.
Yang, D., van, B. S., Reedijk, J., van, B. J., Farrell, N., & Wang, A. H. (1995a) *Nat. Struct. Biol. 2*, 577−586.
Yang, D., van, B. S., Reedijk, J., van, B. J., & Wang, A. H. (1995b) *Biochemistry 34*, 12912−12920.
Zamble, D. B., & Lippard, S. J. (1995) *Trends Biochem. Sci. 20*, 435−439.
Zamble, D. B., Mu, D., Reardon, J. T., Sancar, A., & Lippard, S. J. (1996) *Biochemistry 35*, 10004−10013.
Zorbas, H., Föckler, R., & Winnacker, E.-L. (1990) *Nucleic Acids Res. 18*, 5909−5910.
Zorbas, H., Rogge, L., Meisterenst, M., & Winnacker, E.-L. (1989) *Nucleic Acids Res. 17*, 7735−7748.
Zou, Y., van Houten, B., & Farrell, N. (1993) *Biochemistry 32*, 9632−9638.

BI970741L