Reactions of Unsymmetrically Substituted Derivatives of Cisplatin with Short Oligodeoxynucleotides Containing a -GpG- Sequence: H-Bonding Interactions in pGpG Moieties Cross-Linked by an Asymmetric Platinum Complex Enhancing the Formation of One Geometrical Isomer

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The interaction of unsymmetrically substituted cisplatin derivatives [general formula *cis*-Pt(LL'), L = NH_3: L' = CH_3NH_2 (Pt-mma); L' = $CH_3CH_2NH_2$ (Pt-mea); L' = $NH(CH_3)_2$ (Pt-dma)] with d(GpG), d(pGpG), and d(CpGpG) has been studied by ^1H and ^{31}P NMR spectroscopy. The formation of two geometrical isomers, which were proven to be GN7,GN7 chelates, is slightly influenced by the presence of a 5'-phosphate group and a C base, enhancing the formation of the geometrical isomer with the NH_3 ligand located cis to the 5'G base, thus allowing H bonding toward the 5'-phosphate group. This is only the second clearly performed study demonstrating a correlation between ^{31}P shifts and H bonding. The complexation rate of *cis*-$PtCl_2(NH_3)(Am)$ toward d(GpG) follows the order $CH_3NH_2 > NH_3 \geq CH_3CH_2NH_2 > (CH_3)_2NH$. In addition cytotoxicity data of various human tumor cell lines for these asymmetric Pt amine complexes are reported and compared with cisplatin and carboplatin.

Introduction

The observed antitumor activity of Pt(II) complexes, such as *cis*-diamminedichloroplatinum(II) (cDDP), containing cis amines has prompted studies that demonstrate the need for at least one NH on each amine for reasonable activity.[1] The role such NH groups play in drug binding to DNA, the likely molecular target, is still not completely understood. Both thermodynamic stability and kinetics of complexation have been employed to explain the strong preference of Pt(II) to adjacent guanine N7-sites.[1]

Earlier studies have indicated that the activity of symmetrically substituted platinum complexes (empirical formula *cis*-Pt-(amine)$_2Cl_2$) decreases along the series $NH_3 > RNH_2 > R_2NH > R_3N$ (R representing an alkyl substituent).[3] Apart from steric hindrances,[4] the reactivity of these Pt(II) ions can be influenced by other factors, such as H-bonding ability of the amine ligands,[5] alterations in their trans as well as cis effects,[6] or cell permeability of these Pt compounds.[7]

Reactions of symmetrically or unsymmetrically substituted cis platinum amine compounds with r(GpG) and r(ApG) have recently been described by Alink et al.[8] As a result of the alkyl substituent, both kinetics of complexation and the resulting structure of the adduct are markedly influenced. In particular for secondary amine derivatives slower kinetics are observed. For unsymmetrically substituted Pt complexes no binding selectivity for either the cis or trans side to the substituted amine was found in the first binding step. For the bridged unsymmetric Pt complex Pt(*R*,*S*-dach)Cl_2 (*R*,*S*-dach = 1*R*,2*S*-cyclohexanediamine) this lack of binding selectivity has already been reported in the reaction with d(GpG).[9] However, reaction of the Pt-dach complex with DNA favors formation of one of the stereoisomers almost 2:1, and with Pt(1,3-dach)Cl_2, the binding selectivity is even more pronounced.[10] In addition, binding selectivity was also reported for the interaction of the bridged unsymmetric complex Pt(dmen)-Cl_2 (dmen = *N*,*N*-dimethylethylenediamine) with tetranucleotides.[11]

In a search for new Pt compounds with superior DNA binding properties, a study has been undertaken toward the binding of nonsymmetric Pt complexes to small DNA fragments containing the GG sequence. In the present study the interaction of unsymmetrically substituted derivatives of cDDP with small nucleic acid fragments is described. In order to study adducts representing platinum-DNA adducts, deoxyribonucleotides containing a GpG-site are used (i.e. d(GpG), d(pGpG), and d(CpGpG)). The used cDDP analogues and their abbreviations are depicted in Figure 1.

Both di- and trinucleotides are used as a model for Pt-DNA interactions since the presence of an additional C-base and/or the extra 5'-phosphate might enhance the binding selectivity of the unsymmetrically substituted cDDP derivatives. The first part of this study describes the characterization of the reaction products by ^1H and ^{31}P NMR. The second part deals with competition reaction kinetics between cDDP and an unsymmetric Pt complex toward d(GpG). In addition, cytotoxicity data of the unsymmetric Pt complexes are reported.

Experimental Section

Starting Materials. The deoxynucleotides d(GpG), d(pGpG), and d(CpGpG) were synthesized via an improved phosphotriester method

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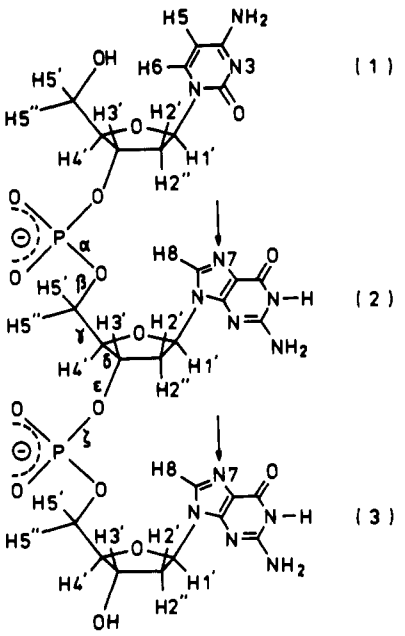

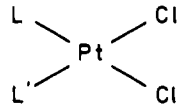

$L = NH_3$	$L' = NH_3$	cisplatin
$L = NH_3$	$L' = NH_2CH_3$	Pt-mma
$L = NH_3$	$L' = NH_2CH_2CH_3$	Pt-mea
$L = NH_3$	$L' = NH(CH_3)_2$	Pt-dma

Figure 1. Schematic representation of d(CpGpG) and structure of the used platinum amine complexes and abbreviations.

and used as their sodium salts.[12] *cis*-$[PtCl_2(NH_3)_2]$ was prepared according to Dhara.[13] The unsymmetric complexes Pt–mma, Pt–mea, and Pt–dma were synthesized as described by Rochon and Kong.[14] Purity was checked by infrared and ^1H NMR spectroscopy. Pt–mma: ^1H NMR –0.76 (s, 3 H) with a broad ^{195}Pt satellite doublet ($^3J(^{195}Pt-^1H) = 24$ Hz). Pt–mea: ^1H NMR –0.45 ppm (q, 2 H), –1.92 ppm (t, 3 H). Pt–dma: ^1H NMR –0.56 ppm (d, 6 H) with a broad ^{195}Pt satellite doublet ($^3J(^{195}Pt-H) = 22.5$ Hz).

Reactions. The oligonucleotide (i.e. d(GpG), d(pGpG), or d(CpGpG)) was dissolved in deionized water at a concentration of 0.5–1.0 mM. One equivalent of the platinum complex was readily dissolved in H_2O after rigorous stirring for a few minutes and added to the nucleotide solution. The pH was adjusted to 5.5 by adding a small amount of NaOH (0.1 M) and the solution was kept in the dark at 310 K for 5 days (pH had dropped to 4). After removal of the solvent, the reaction products were dissolved in 0.5 mL of D_2O (99.8%, Merck) and the samples were lyophilized. The same procedure was followed for the competition reaction between cDDP and the unsymmetric Pt complex towrd d(GpG).

Instrumentation. (a) NMR Spectroscopy. Spectra were recorded on a Bruker WM 300 spectrometer equipped with a variable-temperature unit. The lyophilized products were dissolved in D_2O (99.95%, Merck). ^1H NMR data were collected at 297 and 310 K with TMA (tetramethylammonium nitrate, 3.18 ppm downfield from TMS) used as an internal reference.[15] ^{31}P NMR spectra were obtained at 297 K and referenced to TMP (trimethyl phosphate). The pH dependence of the chemical shift of several nuclei (H8, alkyl substituent, phosphate) was monitored by adding trace amounts of DCl and NaOD (0, 1, and 1.0 M). The pH has not been corrected for deuterium isotope effects.[16]

(b) HPLC. The products of the reaction with the trinucleotide d(CpGpG) were separated and purified using a reversed phase HPLC column (Cosmosil $5C_{18}$), eluted with a linear gradient from 0–30% methanol at 1% min^{-1} against 0.05 M phosphate buffer (pH 8.1). The products was desalted by gel permeation chromatography (Sephadex G-10, Pharmacia), using 0.02 M triethylammonium hydrogen carbonate (TEAB) as eluent. TEAB, a volatile salt, was removed by repeated evaporation with a few drops of NH_4OH.

In Vitro Cytotoxicity. The drugs were tested for their capacity to inhibit the growth of human tumor cell lines using a quantitative propidium iodide staining technique.[17] The method is similar to the MTT assay with the exception that propidium iodide is used instead of a tetrazolium salt to quantify the number of cells. The following cell lines were used: A204 rhabdomyosarcoma; MCF-7 mammary carcinoma; T24 bladder tumor; WiDr colon tumor; IGR-37 melanoma; HT29 colon tumor; A2780 ovarian tumor. For each drug, a dose-response curve was obtained and the ID_{50} value (the drug concentration at 50% inhibition of cell growth) was calculated.

Table I. Ratio of the Two Geometrical Isomers Formed upon Reaction of Unsymmetric Pt Complexes with d(pGpG) or d(CpGpG)

N7,N7 adduct	ratio of the geometrical isomers[a]
Pt–mma–[d(pGpG)-N7(1),N7(2)]	1.09
Pt–mea–[d(pGpG)-N7(1),N7(2)]	1.04
Pt–dma–[d(pGpG)-N7(1),N7(2)]	1.16
Pt–mma–[d(CpGpG)-N7(2),N7(3)]	1.45
Pt–mea–[d(CpGpG)-N7(2),N7(3)]	1.26
Pt–dma–[d(CpGpG)-N7(2),N7(3)]	1.29

[a] Experimental error of alkyl integrals 5%.

Results

I. Identification of the Reaction Products. A. ^1H NMR Spectroscopy. d(GpG). After reaction of d(GpG) with an unsymmetric platinum complex, four H8 signals are observed, 0.2–0.9 ppm downfield compared to free d(GpG). The pH dependence of the base proton chemical shifts showed no N7 protonation effect around pH 2–3, whereas a clear N1 deprotonation effect is observed around pH 8.5 (data not shown).[18] In addition, the coupling pattern of one H1′ changes from a triplet to a doublet, known to be caused by a drastic change in the conformation of the 5′-deoxyribose ring, resulting in a 100% N conformer.[19] All these aspects confirm that both end products are N2,N7 chelates, being geometrical isomers since the platinum complexes lack C_2 symmetry. No preference for the formation of one isomer was found in the case of d(GpG) (i.e., the ratio determined by integration of alkyl resonances was found to be 1.0 ± 0.05). The alkyl chemical shift of one isomer proved to be more pH dependent compared to the other; i.e., at neutral and acidic pH the alkyl resonances of the two geometrical adducts overlap whereas after N1 deprotonation the alkyl signals of the adducts can be observed separately (data not shown).

d(pGpG). The reaction of an unsymmetric Pt complex with d(pGpG) results in two N7,N7 adducts which proved to be geometrical isomers (vide supra). Two H8 signals show two successive titrations of which the first one (pK_a 7) corresponds to the titration of the free 5′-phosphate group, as was also reported for the cDDP adduct[20] and can thus be assigned to the 5′-G H8. The second titration (pK 8.5) corresponding to N1 deprotonation is observed for all H8 resonances. In the case of d(pGpG), binding selectivity is observed; i.e., the ratio of the stereoisomers is not 1:1 (see Table I), although the directing influence of the 5′-phosphate on the isomer ratio is very small. Only for Pt–dma is the adduct ratio seriously influenced by the presence of the additional 5′-phosphate (ratio 1.16), whereas for Pt–mea the ratio is 1:1 i.e., within the experimental error of 5%.

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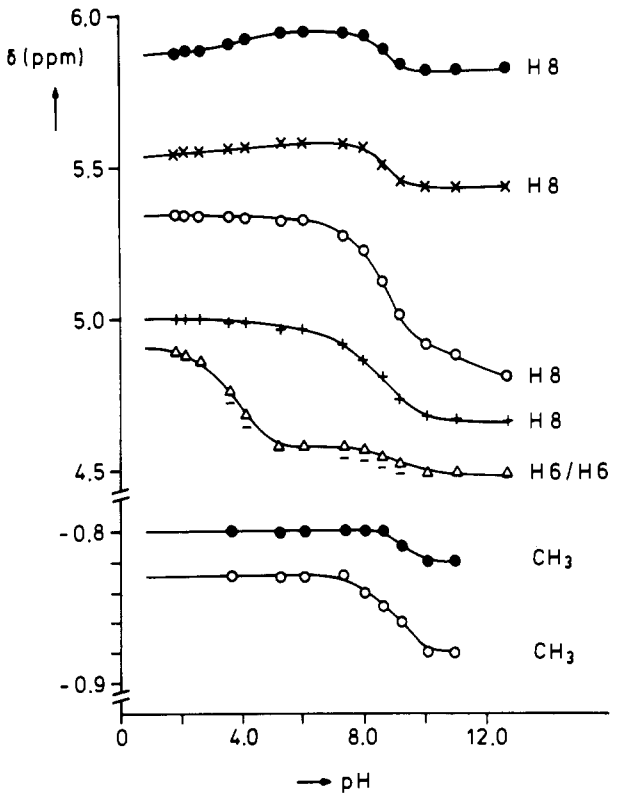

Figure 2. pH versus chemical shift profile of the H8/H6/methyl signals of d(CpGpG)–*cis*-Pt(NH_3)(NH_2CH_3), recorded at 310 K.

d(CpGpG). The G-H8 signals are shifted downfield after reaction of d(CpGpG) with Pt–mma, Pt–mea, or Pt–dma, whereas the H6 signals of the C-base are only slightly influenced. The pH dependence of the base proton chemical shift and of the alkyl substituent for the Pt–mma adducts is depicted in Figure 2. No N7-protonation is observed around pH 2–3 whereas N3-protonation of the C-base still can take place (pK_a 4.2[21]). For d(CpGpG), the ratio of the stereoisomers is seriously influenced (as is depicted in Table I).

It is interesting to note the influence of the N1-deprotonation (pK_a 8.5) upon the methyl chemical shift of the two stereoisomers (Figure 2), the methyl signal of product **1** (major product) being more sensitive than the methyl signal of product **2** (minor product). This might be due to the right-handed helical arrangement of the guanine bases. In the case of product **1**, such an arrangement would be expected to facilitate a hydrogen bond between the NH_3 cis to the 5′-G and the 5′-phosphate (for assignment see also ^{31}P NMR data). This results in a short distance between the alkyl substituent and the O6 of the 3′-G, making the alkyl chemical shift sensitive upon N1-deprotonation of the G-base. On the other hand, in the case of product **2**, the NH_3 is oriented closely to the O6 of the 3′-G and the right-handed helical arrangement leads to a longer distance between the alkylamino group and the O6 of the 5′-G, resulting in a little pH sensitivity for this group, compared to product **1**.

B. ^{31}P NMR Spectroscopy. General Aspects. A relationship between ^{31}P shift and potential hydrogen-bonding interactions in pGpG moieties cross-linked by platinum has been described recently.[11] It was found that the shift of the downfield ^{31}P NMR signals of the GpG moiety can be correlated with the potential H-bonding ability of the platinum moiety and of the oligonucleotide. In particular, if there is a phosphate group 5′ to the GpG unit, the ^{31}P NMR signal is further downfield than in analogous species lacking such a group. Furthermore, when the amine group coordinated cis to the 5′-G is capable of H-bonding (e.g. NH_3), the GpG ^{31}P signal is further downfield than when this group cis to amines has reduced or no H-bonding ability (e.g. $NH(CH_3)_2$).

Table II. ^{31}P NMR Data (ppm) for d(GpG), d(pGpG), and d(CpGpG) and Their Pt Adducts

	pH	–GpG	(C)pG–
d(GpG)	6.07	–4.04	
Pt–mma	6.01	–3.27	
Pt–Mea	5.85	–3.27	
Pt–dma	6.07	–3.30	
cDDP[a]	6.50	–3.35	
d(pGpG)	5.92	–4.04	–2.18
	7.53	–3.98	0.42
	9.06	–3.95	0.80
Pt–mma	6.0	–3.11, 3.65	–1.94, –2.02
	7.5	–2.81, –3.21	0.48
	9.1	–2.90, –3.19	0.86, 0.80
Pt–mea	6.0	–3.10, –3.80	–2.31, –2.46
	7.5	–2.82, –3.33	0.60, 0.52
	9.0	–2.86, –3.21	0.86, 0.81
Pt–dma	6.0	–2.93, –4.10	–2.25, –2.47
	7.5	–2.75, –3.69	0.37
	9.0	–2.90, –3.46	0.81
d(CpGpG)	6.0	–3.85	–4.27
Pt–mma	6.0	–2.62, –3.06	–4.05
Pt–mea	6.0	–2.62, –3.02	–4.02
Pt–dma	6.0	–2.45, –3.15	–4.09
cDDP[b]	6.5	–2.6	–4.1

[a] From ref 19. [b] From ref 31a.

^{31}P NMR of the Geometrical Isomers. For the d(GpG) adducts only one ^{31}P NMR signal is observed around –3.3 ppm, 0.7 ppm downfield of that for the unplatinated d(GpG) (see Table II). This downfield shift is similar to that of d(GpG)–*cis*-Pt(NH_3)$_2$ as reported by den Hartog[19] and is characteristic for cisplatin–GG–N7,N7 chelates.

The ^{31}P spectrum of d(pGpG) of pH 6 consists of one signal at –2.18 ppm, typical of a phosphate monoester, and one phosphodiester signal at –4.04 ppm.[22] After coordination of an unsymmetric Pt complex, four ^{31}P NMR signals are observed for the adducts. The 5′- phosphate signals are strongly affected by pH and shift from –2.1 (pH 6) downfield to 0.80 ppm (pH 9). Two ^{31}P phosphodiester signals are found for the Pt–mma, Pt–mea, and Pt–dma adducts (see Figure 3).

The most downfield phosphodiester signal (–2.9 ppm) is a typical shift observed in a series of Pt adducts in which H bonding of a 5′-phosphate group to an amine ligand of Pt takes place;[11] i.e., this downfield phosphodiester signal must correspond to the geometrical isomer in which the NH_3 ligand is oriented cis toward the 5′-G. The more upfield ^{31}P signal (–3.19 ppm for the Pt–mma adduct, –3.21 ppm for the Pt–mea adduct, and –3.46 ppm for the Pt–dma adduct) is characteristic for GG adducts with less or no H bonding to the 5′-phosphate group[11] and can thus be assigned to the stereoisomer with the alkyl substituent cis to the 5′-G.

The ^{31}P NMR spectrum of d(CpGpG) consists of a CpG signal at –4.27 ppm and a GpG signal at –3.85 ppm. After coordination of the unsymmetric Pt complex, the CpG signal (–4.1 ppm) is affected only slightly (see Table II). Two downfield ^{31}P signals are observed for the phosphodiester group between the G residues of a mixture of the two stereoisomers.

After separation of the two geometrical isomers by HPLC (see Figure 4), the ^{31}P NMR spectra were recorded. The major product exhibits the most deshielded phosphodiester signal (–2.6 ppm). This signal corresponds to the isomer with the alkyl substituent cis to the 3′-G residue, allowing 5′-phosphate group

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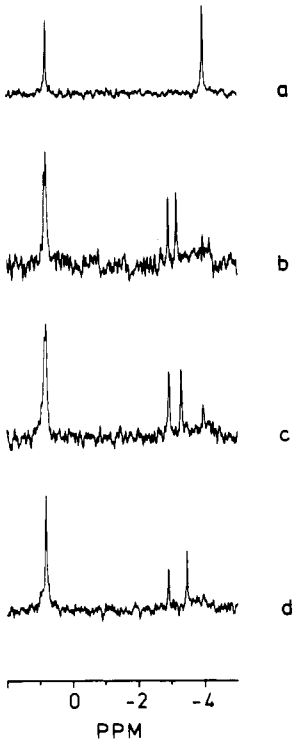

Figure 3. ^{31}P NMR spectra of (a) d(pGpG), (b) d(pGpG)–Pt–mma, (c) d(pGpG)–Pt–mea, and (d) d(pGpG)–Pt–dma at pH 9 and T = 297 K.

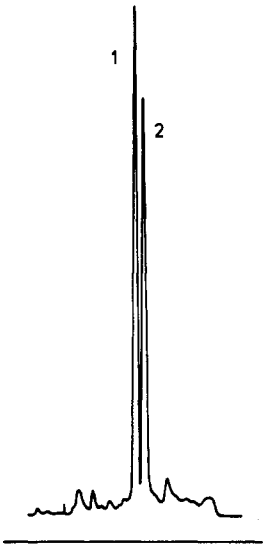

Figure 4. Reversed-phase C18 HPLC separation of the two geometrical isomers of Pt–dma{d(CpGpG),N7(2)N7(3)} (major product (1), minor product (2)).

H bonding to protons of the amine (vide supra). A relatively small downfield shift is found for the GpG signal of the minor product (ca. –3.1 ppm) which can thus be assigned to the stereoisomer with the alkyl substituent cis to the 5′-G (see Figure 5).

II. Competititon Reactions. The competition reactions between [*cis*-$PtCl_2(NH_3)_2$] and the unsymmetric Pt compounds toward d(GpG) proved to be completed within 1 week (at 37 °C), as no unreacted d(GpG) was observed anymore. Using the data obtained for the stereoisomers (part A) and the NMR data previously reported for *cis*-$Pt(NH_3)_2$[d(GpG)-N7(1),N7(2)],[20] the products could be assigned unambiguously, being d(GpG) chelates of either cDDP or an unsymmetric Pt complex. The relative amounts of the adducts have been easily determined by integration of the H8 signals. Since H8 protons of platinated guanine residues are susceptible to exchange with deuterium[23] and broad, due to unresolved coupling with the ^{195}Pt nucleus,[24] they are usually not suitable for the observation of accurate integrals. Therefore, as a control, the integrals of the alkyl substituent of the Pt–mma adducts were taken (at high pH) to estimate the relative amount of coordinated unsymmetric Pt complex and thus the percentages of the cDDP adduct. The experimental error was found to be less than 5%, justifying the use of H8 integrals. The results of the competition reactions are summarized in Table III and show the order of reactivity of *cis*-$PtCl_2(NH_3)$(Am) toward d(GpG):

$$CH_3NH_2 > NH_3 \geq CH_3CH_2NH_2 > (CH_3)_2NH$$

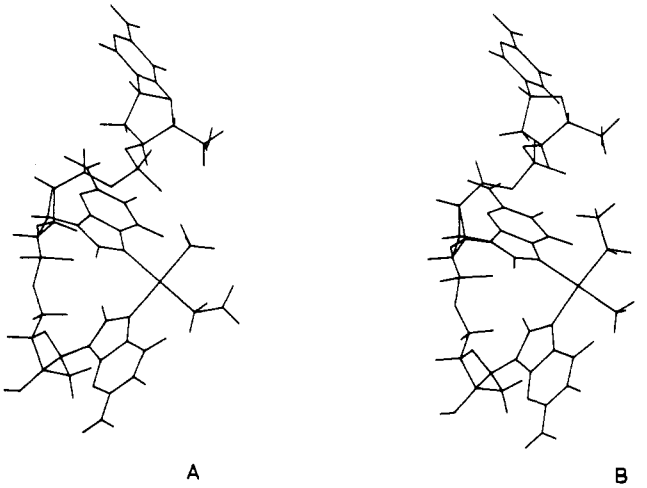

Figure 5. Schematic representation of the two geometrical isomers of Pt–mma–[{d(CpGpG)-N7(1),N7(2)}] with the alkyl substituent cis toward 3′-G (major product, A) and with the alkyl substituent cis toward 5′-G (minor product, B).

Table III. Relative Amont of cDDP Adduct Found in Competition with an Unsymmetric Pt Complex

unsymmetric Pt complex	relative amount of *cis*-$Pt(NH_3)_2$–[d(GpG)-N7(1),N7(2)]
Pt–mma	28%[a] (±1)
	32%[b] (±1)
Pt–mea	56‰[a] (±1)
Pt–dma	>90%[a]

[a] H8 integral. [b] Alkyl integral.

Table IV. ID_{50} Values of Unsymmetric cDDP Derivatives Tested against Human Tumor Cell Lines Using an in VItro Propidium Iodide Staining Technique (ID_{50}) Values in ng/mL

	ID_{50} values						
drug	A204	MCF7	T24	WiDr	IgR-37	HT29	A2780
Pt–mma	653	604	1013	434	1047	302	217
Pt–mea	271	232	970	372	745	180	220
Pt–dma	1118	1424	3393	1440	3665	928	813
cDDP	402	330	381	190	434	122	118
CBDCA	4181	2910	3108	6560	2694		1221

III. Cytotoxicity of the Platinum Compounds. The results of the in vitro assay are summarized in Table IV. The ID_{50} values of the unsymmetric platinum compounds are almost compatible to the ID_{50} value of cisplatin, Pt–dma being less active compared to Pt–mma and Pt–mea. A recent study with leukemia L1210 shows that unsymmetric Pt compounds with a primary substituted amine ligand have lower ID_{50} values, compared to the corre-

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sponding complexes with secondary or tertiary amino ligands,[25] in accordance with the data obtained in this study for the solid tumor model.

Discussion

It has been stated recently that platination of DNA is controlled kinetically, rather than thermodynamically.[26] The rate-limiting step of initial binding is hydrolysis of the first chloride ion, after which the complex coordinates to the N7 position of guanine. The binding site of the first platination step, i.e. the monofunctional adduct, controls the ratio of the two geometrical isomers. For d(GpG), binding selectivity for the 5′-G has recently been reported in its reaction with monofunctional platinum compounds.[27] A kinetic study of mixed-amine cDDP-analogues, containing benzylamine and (2-phenylethyl)amine, shows that the two rate constants for the hydrolysis of the first Pt–Cl bond are not equal.[28] Due to the severe steric hinderance of the bulky (alkylaryl) amine group, the trans chloro ligand will be hydrolyzed selectivily, before substitution of the cis chloro ligand occurs. For the unsymmetric Pt complexes described here, the alkyl subsituents are less bulky, compared to the alkylaryl substituents; thus, the rate constants for the hydrolysis of the two Pt–Cl bonds are expected to be almost identical. In fact, reaction of d(GpG) with unsymmetrically substituted cDDP analogues yields the two geometrical isomers in equal amounts.

In reaction with longer nucleotides however, a slight preference for one geometrical isomer is found, which can be ascribed to the differences in hydrogen-bonding ability of the two amine ligands towards the 5′-phosphate group. Such hydrogen bridges appear to be important for the Pt–DNA interaction, both kinetically, i.e. enhancing platinum coordination, and thermodynamically, i.e. stabilization of the adduct. Kinetic studies with small oligonucleotides show that platination of the guanine base is enhanced by the presence of a 5′-phosphate.[5a,20,29] The X-ray structures of *cis*-$[Pt(NH_3)_2\{d(pGpG)\}]$ and *cis*-$[Pt(NH_3)_2\{d(CpGpG)\}]$[30] provide evidence for an intramolecular ammine···phosphate hydrogen bond, although for the d(CpGpG) adduct this bond appears to be longer (probably due to destacking of the adjacent C-base). In solution, however, the C-base stacks well on top of G(2) and a large downfield shift for the interguanine phosphorus resonance has been observed in the ^{31}P NMR spectra of *cis*-$[Pt(NH_3)_2\{d(CpGpG)\}]$ and longer oligonucleotides containing the intrastrand d(GpG) cross-link, characteristic for the presence of a NH_3···phosphate bond.[11,31] This study confirms and extends the NMR results of Spellmeyer-Fouts et al. demonstrating a correlation between ^{31}P shifts and H-bonding.[11]

Very recently, we became aware of the work of Hartwig et al.[32] describing the DNA-binding properties of the orally active platinum anticancer drug *cis,trans,cis*-$[Pt(NH_3)(C_6H_{11}NH_2)(OC(O)C_3H_7)_2Cl_2]$. The two geometrical isomers of *cis*-$[Pt(NH_3)(C_6H_{11}NH_2)\{d(GpG)\text{-}N7(1),N7(2)\}]$, corresponding to the 3′ versus the 5′ orientation of the cyclohexylamine ligand, were observed for platination of DNA (2:1 ratio), as well as shorter oligos (4:3 ratio). The orientational isomer having the cyclohexyl group directed toward the 3′ end of the platinated strand was identified as the more abundant of the two geometrical isomers, in accordance with our results. Both isomers were proven to be less efficient than cisplatin in blocking replication. In addition each isomer inhibits DNA synthesis at different sites and to slightly different degrees, probably due to the different abilities of the two isomers to form a hydrogen bond toward the 5′ phosphate.

Surprisingly, Pt–mma proves to be slightly more reactive toward d(GpG) compared to cisplatin, although the methyl group in tightly bound amines would be expected to form a steric obstacle for the incoming G base. Arpalahti et al. recently observed that the complexation rate of various aquated cis Pt(II) diamines with purine nucleosides follows the order $CH_3NH_2 > NH_3 > (CH_3)_2NH$ in a slightly acidic medium. The enhanced reactivity of *cis*-$[Pt(mma)_2(H_2O)_2]^{2+}$ has been ascribed to the labilization of the coordinated water molecule as a consequence of methyl substitution.[6] This order of reactivity is similar to the results obtained for the competition reaction with d(GpG). However, one should realize that for the unsymmetric Pt compounds the overall order of reactivity is reported, including hydrolysis of the first Pt–Cl bond, coordination to GN7, subsequent hydrolysis of the second chloride ion, and finally reaction with a second base, resulting in the GG-N7,N7 adduct. A detailed study determining the two Pt–Cl hydrolysis rate constants of the unsymmetric Pt complexes according to the method of Bednarsky et al.,[28] and in addition the kinetics of the first binding step and the chelation step, using reversed-phase HPLC, would be required to explain these observations in further detail.

In reaction of cisplatin derivatives with r(ApG) the order of reactivity proved to be $NH_3 > RNH_2 > R_2NH$.[8a] This deviation from the results with d(GpG) can be understood considering the steric effects suggested earlier for the C6 NH_2 group, i.e. the amino group at C6 sterically prevents the attack of Pt(II), whereas with the oxo substituent enhancement of the reaction rate can be attributed to the formation of an H bond from the coordinated water molecule to O6 in both binding steps.[5a,6]

The cytotoxicity data reveal that platinum complexes in which one of the two nonleaving ligands is substituted by a primary amine ligand show ID_{50} values lower than the corresponding complexes with a secondary amine ligand. For leukemia L1210, it was reportd that the amount of Pt uptake is not significantly affected by the alkylamine substitution.[25] Therefore, it is unlikely that the amount of Pt uptake is responsible for the different ID_{50} values. One should thus consider the binding mode with DNA. The presence of the alkyl groups on the ligand L′ does influence the kinetics of formation of the bifunctional adducts; i.e., the bulky group in Pt–dma is likely to retard the initial binding step (due to steric hinderance and a decrease in H-bonding ability toward DNA) and in addition is likely to prolong the lifetime of the monofunctional intermediates (due to slower rotation around the platinum–N7 bond), making these more reactive towards S-containing nucleophiles such as glutathione and other intracellular nucleophiles. These agents can bind at the labile site of monofunctional adducts and prevent ring closure or can act as an inactivator of Pt complexes, thereby preventing the overall binding to DNA.[26,33] All these effects may account for the lower cytotoxicity of Pt–dma.

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Concluding Remarks

The reaction of unsymmetrically substituted cisplatin analogues with d(GpG) results in two GN7,GN7 adducts, which prove to be geometrical isomers. The modification by addition of a 5′-phosphate group or a 5′C-base to the GG fragment induces a slight preference for the formation of the stereoisomer with the NH_3 ligand cis towards the 5′-G (allowing H bonding toward the 5′-phosphate). The alkyl substituents do influence the reaction velocity towards d(GpG). In particular, in the case of Pt–dma, slow kinetics are observed and the low antitumor activity of cisplatin analogues containing bulky nonleaving groups might well be related to the slow kinetics of binding to DNA. It will be of interest to determine the ratio of the geometrical isomers in their reaction with DNA and whether or not the two geometrical isomers contribute to the same extent to the in vitro cytotoxicity, since for the two geometrical isomers of the adduct *cis*-[$Pt(NH_3)(C_6H_{11}NH_2)${d(GpG)-N7(1),N7(2)}] replication inhibition occurs with different efficiencies depending on the 3′ or 5′ orientation of the alkylamine ligand.

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