Interaction of Cisplatin and DNA-Targeted 9-Aminoacridine Platinum Complexes with DNA†

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ABSTRACT: Interaction of acridine- and 9-aminoacridinecarboxamide platinum complexes with DNA was investigated with respect to their DNA sequence specificity and kinetics of binding. The DNA sequence specificity of the compounds was quantitatively analyzed using a polymerase stop assay with the plasmid pUC19. The 9-aminoacridinecarboxamide platinum complexes exhibited a different sequence specificity to that of cisplatin, shifted away from runs of consecutive guanines (the main binding site for cisplatin). This alteration was dependent on chain length. Shorter chain length compounds ($n = 2, 3$) showed a greater difference in sequence specificity, while longer chain length compounds ($n = 4, 5$) more closely resembled cisplatin. An acridinecarboxamide platinum complex showed a similar sequence specificity to cisplatin, revealing that the major change of sequence specificity was due to the presence of the 9-amino substituent. A linear amplification system was used to investigate the time course of the reaction. The presence of an intercalating group (acridinecarboxamide or 9-aminoacridinecarboxamide) greatly increased the rate of reaction with DNA; this is proposed to be due to a different reaction mechanism with DNA (direct displacement by the N-7 of guanine).

Cisplatin[1] is clinically used as a cancer chemotherapeutic agent (*1*). Cisplatin reacts with DNA inside cells forming cross-links (*2, 3*). DNA intrastrand cross-links at GG sequences are thought to be the crucial lesion for its biological effectiveness as an antitumor agent. The main site of adduct formation is at the N-7 of guanine. Sequence specificity studies have shown that adducts form mainly at runs of consecutive guanines, with less intense damage at AG, GA, and GC sequences (*4*−*7*). A similar sequence specificity is found in intact human cells (*8*). Sequence selectivity of platination has been determined for a number of cisplatin analogues in both plasmid (*7, 9, 10*) and human cells (*8, 11*). Until the experiments described in this paper, no study has convincingly shown an altered sequence specificity for a cisplatin analogue.

The sequence specificity of cisplatin and analogues can be determined using a polymerase stop assay—usually the linear amplification reaction (*6*). In this assay, *Taq* DNA polymerase extends from an oligonucleotide primer until inhibited by a cisplatin adduct. The products are linearly amplified by thermal cycling and run on a DNA sequencing gel alongside dideoxy sequencing reactions as size markers. The precise sequences damaged by cisplatin can be determined as well as the relative intensity of damage at each lesion site (*7, 9*).

There have been numerous attempts to improve the effectiveness of cisplatin as an antitumor agent by the modification of its structure (*12*). The approach that has been examined in this paper is the DNA targeting hypothesis (*13*), in which a DNA affinic group is attached to the platinum complex. Cisplatin and the related $PtenCl_2$ molecules have no intrinsic affinity for DNA, and the main hypothesized effect of attachment of a DNA affinic group is to place the platinum close to the biological target, DNA. A number of consequences could flow from this, including the following: reduced side reactions with other molecules in the cell; altered kinetics of binding; different reaction mechanism; and possibly an altered sequence specificity of covalent binding. A different sequence specificity might result in a different spectrum of lesions on DNA, including novel lesions that may escape the DNA repair process.

Previously, we have shown an increased rate of reaction with DNA for platinum complexes with an attached phenanthridinium intercalating group (*10*), and a similar observation

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[1] Abbreviations: cisplatin, *cis*-diamminedichloroplatinum(II); $PtenCl_2$, dichloro(ethylenediamine)platinum(II); $PtenCl_2$(n2), dichloro(*N*-ethylethylenediamine)platinum(II); $PtenCl_2$(n3), dichloro(*N*-propylethylenediamine)platinum(II); ac-$PtenCl_2$(n3), dichloro(*N*-[3-[(2-aminoethyl)amino]propyl]acridine-4-carboxamide)platinum(II); 9-aminoac-$PtenCl_2$(n2), dichloro(*N*-[2-[(2-aminoethyl)amino]ethyl]-9-aminoacridine-4-carboxamide)platinum(II); 9-aminoac-$PtenCl_2$(n3), dichloro(*N*-[3-[(2-aminoethyl)amino]propyl]-9-aminoacridine-4-carboxamide)platinum(II); 9-aminoac-$PtenCl_2$(n4), dichloro(*N*-[4-[(2-aminoethyl)amino]butyl]-9-aminoacridine-4-carboxamide)platinum(II); 9-aminoac-$PtenCl_2$(n5), dichloro(*N*-[5-[(2-aminoethyl)amino]pentyl]-9-aminoacridine-4-carboxamide)-platinum(II); 9-aminoac(n2), *N*-[2-[(2-aminoethyl)amino]ethyl]-9-aminoacridine-4-carboxamide; 9-aminoac(n3), *N*-[3-[(2-aminoethyl)amino]-propyl]-9-aminoacridine-4-carboxamide; 9-aminoac(n4), *N*-[4-[(2-aminoethyl)amino]butyl]-9-aminoacridine-4-carboxamide; 9-aminoac(n5), *N*-[5-[(2-aminoethyl)amino]pentyl]-9-aminoacridine-4-carboxamide.

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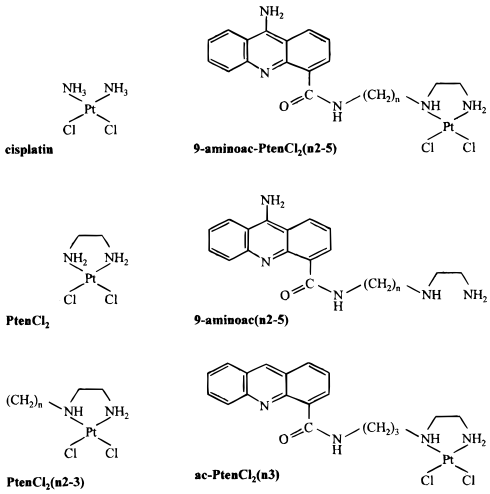

FIGURE 1: Structures of cisplatin and related complexes used in this study.

has also been made for phenazine-tethered platinum complexes (*14*). In both cases, it was postulated that the altered kinetics, when compared with cisplatin, might be due to a different reaction mechanism. Instead of the chloride leaving group being replaced by H_2O before reaction with DNA (a slow step with $t_{1/2}$ of 2 h), the chloride could be directly displaced by the N-7 of guanine. This direct displacement model can occur because the intercalating group places the platinum close to the N-7 of guanine.

In this paper, we show an altered DNA sequence specificity for a series of 9-aminoacridinecarboxamide platinum complexes. We describe the sequence specificity and relative time course of adduct formation of eight platinum complexes and compare their behavior with that of cisplatin (see Figure 1 for structures). The following five groups of compounds were examined:

(1) the reference control compound, cisplatin;

(2) 9-aminoacridinecarboxamide platinum complexes, the main subject of this study;

(3) acridinecarboxamide platinum complex;

(4) the metal-free 9-aminoacridine ligands;

(5) the platinum complexes of the ligands ethylenediamine, *N*-ethylethylenediamine, and *N*-propylethylenediamine (lacking an attached intercalator). These are closer structural analogues of the platinum portion of the intercalator-tethered complexes than cisplatin.

Groups 1, 3, 4, and 5 were control compounds. Group 5 [$PtenCl_2$, $PtenCl_2$ ($n = 2, 3$)] was used to investigate the influence of the intercalator. Group 4 [9-aminoacridine ($n = 2, 3, 4, 5$)] examined the effect of platinum. The sequence specificity of the group 3 compound, acridinecarboxamide ($n = 3$) platinum complex, has been previously determined (*7*); this was included as a control to examine the effect of the 9-amino substituent. The compounds in group 2 were the main subject of study and comprised four 9-aminoacridinecarboxamide platinum complexes containing polymethylene chains of different length (2, 3, 4, and 5 carbons) separating the intercalator and the platinum complex.

MATERIALS AND METHODS

Materials. Cisplatin, dichloro(ethylenediamine)platinum(II) ($PtenCl_2$), dichloro(*N*-ethylethylenediamine)platinum(II) [$PtenCl_2$ (n2)], and dichloro(*N*-propylethylenediamine)platinum(II) [$PtenCl_2$ (n3)] were prepared following the method of Dhara (*15*). The complexes dichloro(*N*-[3-[(2-aminoethyl)-amino]propyl] acridine-4-carboxamide)platinum(II) [ac-$PtenCl_2$(n3)] and dichloro(*N*-[3-[(2-aminoethyl)amino]propyl]-9-aminoacridine-4-carboxamide)platinum(II) [9-aminoac-$PtenCl_2$(n3)] were prepared using methods we have previously reported (*7*). The same general methods were used to prepare the new ligands *N*-[2-[(2-aminoethyl)amino]ethyl]-9-aminoacridine-4-carboxamide [9-aminoac(n2)], *N*-[4-[(2-aminoethyl)amino]butyl]-9-aminoacridine-4-carboxamide [9-aminoac(n4)], and *N*-[5-[(2-aminoethyl)amino]pentyl]-9-aminoacridine-4-carboxamide [9-aminoac(n5)] and the platinum complexes dichloro(*N*-[2-[(2-aminoethyl)amino]ethyl]-9-aminoacridine-4-carboxamide)platinum(II) [9-aminoac-$PtenCl_2$-(n2)], *N*-[4-[(2-aminoethyl)amino]butyl]-9-aminoacridine-4-carboxamide [9-aminoac-$PtenCl_2$(n4)], and *N*-[5-[(2-aminoethyl)amino]pentyl]-9-aminoacridine-4-carboxamide [9-aminoac-$PtenCl_2$(n5)]. All compounds were characterized by elemental analysis; ^1H, ^{13}C, and ^{195}Pt NMR; and electrospray ionization mass spectrometry. Full synthetic details for new compounds will be reported elsewhere.

All compounds were dissolved in dimethylformamide to give stock solutions of 1 mM. The plasmid pUC19 was prepared by a heat-alkali method (*16*).

DNA Damage Experiments. Sequence specificity of adduct formation was determined by treating 5 μg of pUC19 plasmid DNA with a final concentration of 0.05−0.5 μM cisplatin or platinum complex for 18 h. All damage reactions were maintained in darkness at 37 °C in 2 mM HEPES, pH 7.8, 10 mM NaCl, and 10 μM EDTA in a final reaction mix of 40 μL. After ethanol precipitation (4.4 μL of 3 M sodium acetate, 88.8 μL of ethanol) and two ethanol washes were performed, the DNA was dissolved in 20 μL of 10 mM Tris-HCl, pH 8.5, and 100 μM EDTA.

The time course of adduct formation was determined in a scaled up reaction mix consisting of 40 μL/time point; typically, this consisted of a final reaction mix of 280 μL for 7 time points. For each time point, a 40-μL aliquot was taken from the mix and added to a cold (on ice) stop bath mixture of 4.4 μL of 3 M sodium acetate and 88.8 μL of ethanol to precipitate the DNA and stop the reaction. In the zero time (stopped) control, a 40-μL aliquot was taken from the reaction mix prior to the addition of the cisplatin or analogue and added to the stop bath to precipitate the DNA. The cisplatin or analogue was then added and mixed—this control excluded the possibility of adduct formation during the ethanol precipitation or the linear amplification procedures.

Incubated DNA controls were prepared for all damage reactions. In these, dimethylformamide or H_2O was added in place of a platinum complex. These controls monitor the negligible damage due to solvent effects or extended incubations in the reaction mix.

Linear Amplification Reaction. The linear amplification reaction comprised a mix of 16.6 mM $(NH_4)_2SO_4$; 67 mM Tris-HCl, pH 8.8; 6.7 mM $MgCl_2$; 300 μM each of dATP, dGTP, dCTP, and dTTP; 0.05 pmol of ^{32}P-labeled primer; 0.25 units of Ampli*Taq* DNA polymerase (Perkin-Elmer Cetus) (final concentrations). The sequencing primer utilized was (REV) 5′-AACAGCTATGACCATG-3′ and was 5′-labeled as previously described (*17*) with polynucleotide

kinase (Pharmacia) and [γ-^{32}P]ATP (Amersham). DNA (2 μL) was added to this mix producing a final volume of 5 μL, which was overlaid with 50 μL of mineral oil. Dideoxy double-stranded DNA sequencing was performed using the same sequencing primer to accurately pinpoint the precise site of damage. The linear amplification procedure subjected the reaction mix to 95 °C for 30 s (time at temperature), 50 °C for 60 s, and 72 °C for 90 s for 20 cycles in a Perkin-Elmer Cetus DNA thermal cycler 480. Two microliters of the reaction mix was then electrophoresed on a 6% polyacrylamide-urea DNA sequencing gel. The gel was dried and analyzed by a Molecular Dynamics PhosphorImager after exposure to a phosphor storage screen.

Densitometry. Densitometry was performed using Molecular Dynamics ImageQuaNT v4.2a software. For determination of the sequence specificity of adduct formation, the intensity at all bands was calculated by integration of the peak area of the entire lane, from bp 210 to 440. The baseline of each line graph was determined using the lowest point method, and the boundaries of each site of damage were selected manually. For determination of the time course of adduct formation, the peak area of a damage band was integrated across sequential lanes to give a set of damage intensities, one for each time point. The baseline of each line graph was determined using the lowest point method, and the lane boundary of each damage band was determined manually. Each set of damage intensities was determined after baseline subtraction. For cisplatin and each analogue, the intensities of five damage sites were determined and normalized relative to the time point at which the maximum intensity occurred. The average relative intensities of cisplatin and each complex were plotted against time.

RESULTS

Sequence specificity and time course of adduct formation by cisplatin and related complexes in pUC19 have been evaluated using the linear amplification procedure. The products of these reactions were run on DNA sequencing gels alongside appropriate dideoxy sequencing lanes (Figures2 and 3). The intensity of damage at each site was quantified using densitometry. In all cases, the level of damage in the no drug control lanes was negligible as compared to damage in the drug-treated lanes. The control compounds, 9-aminoac(n2−5) that lack platinum and cannot form platinum adducts, produced no significant damage.

Sequence Specificity. The sites of damage by the platinum complexes can be observed in the DNA sequencing gels shown in Figures 2 and 3. The sequences of the 10 most intense sites of damage are depicted in Table 1. The most intense damage sites of cisplatin in pUC19 are found at a run of five consecutive guanines (bp 318−322) and four guanines (bp 415−418) (Figures 2 and 3). These two runs of guanines are also the most intense sites of damage for the "untargeted" complexes $PtenCl_2$ and $PtenCl_2$(n2,3) and the previously studied (*7*) acridine-linked compound ac-$PtenCl_2$(n3). For the sequences shown in Table 1, these complexes have at least 8 of the 10 damage sequences in common with cisplatin.

However, the 9-aminoac-$PtenCl_2$(n2,3) complexes exhibited striking differences in sequence specificity relative to cisplatin and $PtenCl_2$. The 9-aminoac-$PtenCl_2$(n2,3) complexes did not exhibit a strong preference for adduct formation at sequences containing consecutive runs of guanines. Instead, they had a preference for 5′-tgAat-3′,

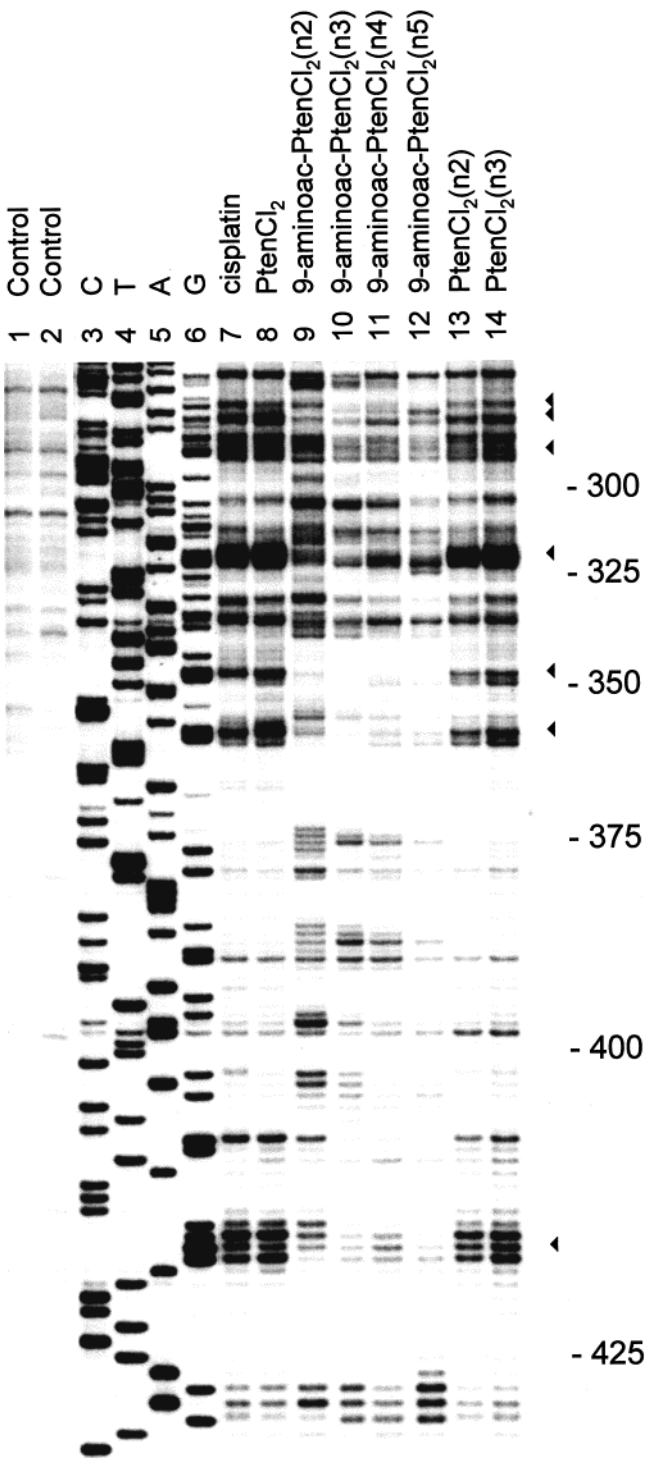

FIGURE 2: Phosphorimage of a DNA sequencing gel comparing the sequence specificity of damage due to cisplatin and its analogues in pUC19. Sequence specificity of adduct formation was determined by treating pUC19 plasmid DNA with 0.15 μM cisplatin or platinum complex for 18 h. All samples were linearly amplified using the REV primer. Lanes 1 and 2 are untreated damage control lanes. Lanes 3−6 are the pUC19 dideoxy sequencing lanes and give the sequence on the template strand. Lanes 7−14 were derived from damage due to cisplatin, $PtenCl_2$, 9-aminoac-$PtenCl_2$(n2), 9-aminoac-$PtenCl_2$(n3), 9-aminoac-$PtenCl_2$(n4), 9-aminoac-$PtenCl_2$(n5), $PtenCl_2$(n2), and $PtenCl_2$(n3), respectively (as indicated). Black arrows indicate the position in the sequence of three or more consecutive G bases.

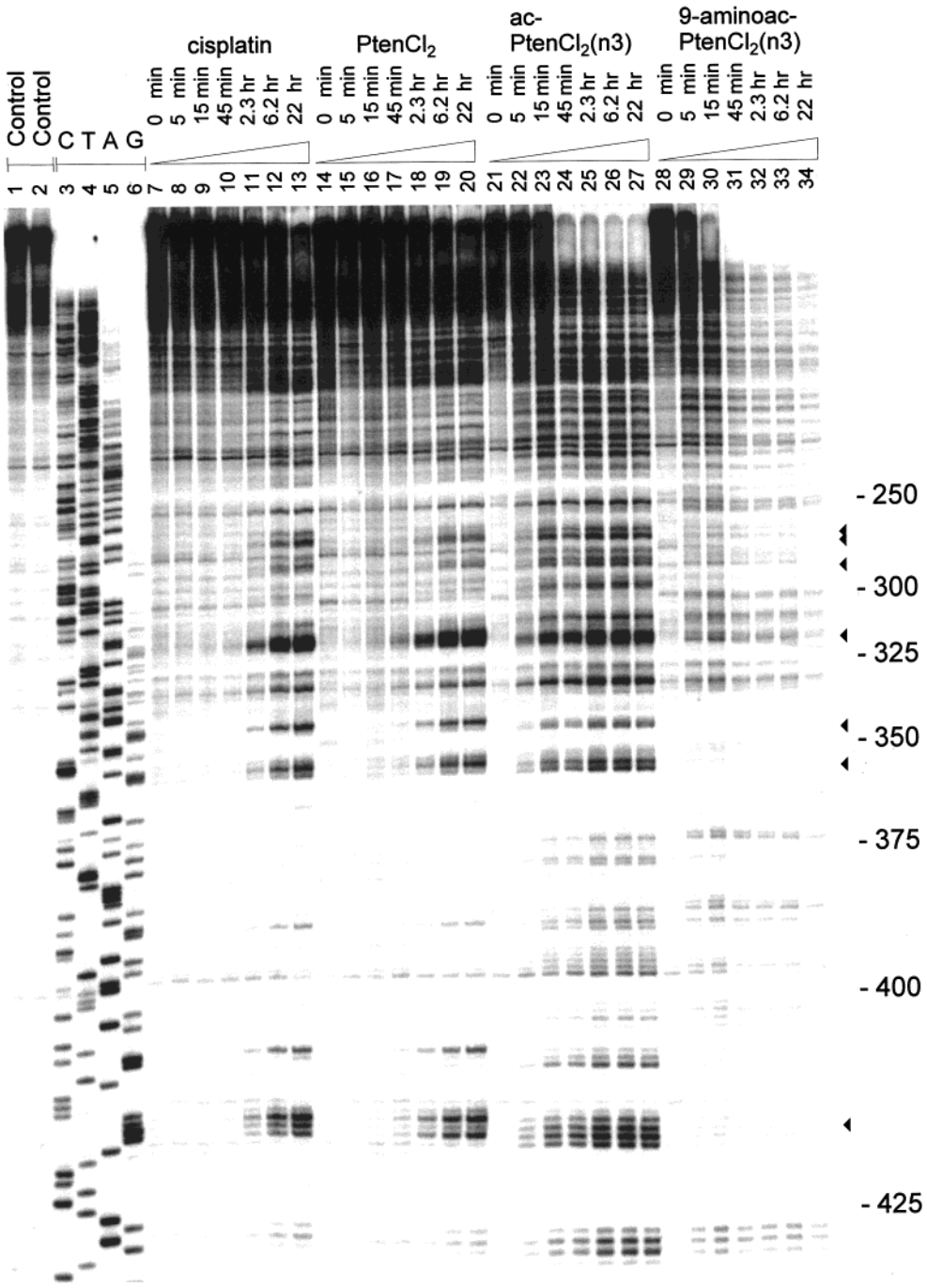

FIGURE 3: Phosphorimage of a DNA sequencing gel comparing the time course of damage due to cisplatin and its analogues in pUC19. All samples were linearly amplified using the REV primer. Lanes 1 and 2 are untreated damage control lanes. Lanes 3−6 are the pUC19 dideoxy sequencing lanes and give the sequence on the template strand. Lanes 7−13 are from damage due to cisplatin (0.15 μM), lanes 14−20 are from $PtenCl_2$ (0.15 μM), lanes 21−27 are from ac-$PtenCl_2$(n3) (0.05 μM), and lanes 28−34 are from 9-aminoac-$PtenCl_2$(n3) (0.15 μM). Incubation times for each compound were 0, 5, 15, and 45 min and 2.3, 6.2, and 22 h. Black arrows indicate the position in the sequence of three or more consecutive G bases.

agAgt, gaCct, acGcc, ctGca, caCga, tgCag, and cgAgc sites (Table 1).

The 9-aminoac-$PtenCl_2$(n2−5) complexes vary in the length of the polymethylene linker chain ($n = 2$, 3, 4, or 5) that tethers the 9-amino-acridinecarboxamide intercalator to the $PtenCl_2$ moiety. The shortest chain ($n = 2$) homologue had the most dissimilar sequence specificity as compared to that of cisplatin. As the linker chain length increased, the sequence specificity became progressively more similar to that of cisplatin. This can be observed in Table 1 for the number of damage sites that contain runs of two or more consecutive guanines. The $n = 2$ compound had only 4 out of 10 damage sites with consecutive guanine, while $n = 3$ had 5 out of 10; $n = 4$ had 7 of out 10; and $n = 5$ had 7 out of 10. Cisplatin and $PtenCl_2$ both had 9 out of 10; ac-$PtenCl_2$-(n3) had 8 out of 10; and $PtenCl_2$(n2,3) had 9 out of 10.

The maximum intensity is shown for each compound in Table 1, with cisplatin having a value of 12 100. For 9-aminoac-$PtenCl_2$(n2−5) complexes, the maximum intensity is lowest for $n = 2$ and gradually increases through $n = 3$, 4, and 5, with the $n = 5$ compound being most similar to cisplatin.

Table 1: DNA Sequence Specificity of Cisplatin and Analogues in pUC19[a]

cisplatin			$PtenCl_2$			ac-$PtenCl_2$(n3)			$PtenCl_2$(n2−3)		
sequence	bp	RI 12100	sequence	bp	RI 12200	sequence	bp	RI 11300	sequence	bp	RI 8700
aggGgga	320	1.00	aggGgga	320	1.00	cccGggg	415	1.00	aggGgga	320	1.00
ccgGgga	416	0.85	ccgGgga	416	0.77	aggGgga	320	0.94	ccgGgga	416	0.75
cagGgtt	358	0.43	cagGgtt	358	0.34	aggCatg	442	0.66	tgcGtaa	213	0.36
cagGcat	441	0.35	aggCatg	442	0.30	tagAgtc	428	0.52	cagGgtt	358	0.34
gaaGggc	273	0.31	gttGggt	346	0.27	gttGgga	268	0.52	gaaGggc	273	0.31
aggCgat	336	0.30	gaaGggc	273	0.27	aggCgat	336	0.50	ttgGgta	347	0.30
ttgGgta	347	0.29	aagGaga	218	0.24	ccaGggt	357	0.47	cagGcat	441	0.30
ctcGgta	408	0.24	cggTacc	410	0.24	tgaAttc	398	0.42	aggCgat	336	0.29
gcgGgcc	287	0.23	aggCgat	336	0.23	gcgGgcc	287	0.41	ctcGgta	408	0.25
tagAgtc	428	0.23	tagAgtc	428	0.18	gaaGggc	273	0.41	aagGaga	218	0.24

9-aminoac-$PtenCl_2$(n2)			9-aminoac-$PtenCl_2$(n3)			9-aminoac-$PtenCl_2$(n4)			9-aminoac-$PtenCl_2$(n5)		
sequence	bp	RI 3600	sequence	bp	RI 4600	sequence	bp	RI 4000	sequence	bp	RI 10400
gtgAatt	397	1.00	tagAgtc	428	1.00	aggGgga	320	1.00	ctaGagt	427	1.00
tagAgtc	428	0.97	cgaCctg	434	0.92	agaGtcg	429	0.89	aggGgga	320	0.52
tacGcca	304	0.87	aggCatg	442	0.84	aggCatg	442	0.86	aagGaga	218	0.36
ccgGgga	416	0.86	cgaCggc	388	0.74	cggGgat	417	0.74	aggCatg	442	0.35
gctGcaa	330	0.84	tcaCgac	372	0.66	ggcGaaa	314	0.63	tgcGtaa	213	0.34
ctgCagg	438	0.82	tacGcca	304	0.65	aagGcga	335	0.60	ggcGaaa	314	0.28
tcgAgct	403	0.81	aggGgga	320	0.59	tgcGtaa	213	0.56	aagGcga	335	0.26
gcaGgca	440	0.80	aagGcga	335	0.55	gctGgcg	311	0.54	gggGatc	418	0.26
aagGcga	335	0.79	ccgGgga	416	0.54	acgGcca	390	0.51	ttgGgaa	269	0.25
aggGgga	320	0.78	ctgCagg	438	0.52	aagGaga	218	0.45	tcaGgct	253	0.20

[a] The most intense damage sites were arranged in decreasing order with respect to the relative intensity (RI) of each compound. The intensity (in arbitrary units) of the most intense site is indicated below the RI heading. The sequences are written from 5′ to 3′ with the capital letter indicating the site of maximum damage for each damage cluster. The pUC19 plasmid sequence investigated extended from bp 210 to bp 440. Note that *Taq* DNA polymerase approached the damage site from the right of the indicated sequence.

Another interesting feature of the 9-aminoac-$PtenCl_2$(n2−5) complexes was the range of damage intensities produced at different sites. The relative intensity (RI) of damage sites for each compound is shown in Table 1. The 10th most intense damage site had an RI of 0.23 for cisplatin but 0.78 for 9-aminoac-$PtenCl_2$ ($n = 2$), 0.52 for $n = 3$, 0.45 for $n = 4$, and 0.20 for $n = 5$. This indicates that the 9-aminoacridine platinum compounds $n = 2$, 3, and 4 had a large number of damage sites with very similar damage intensities. Again, a correlation is observed between linker length and cisplatin-like properties, i.e., as linker length increases, the behavior of the 9-aminoac-$PtenCl_2$(n2−5) compounds became more similar to that of cisplatin and $PtenCl_2$. The ac-$PtenCl_2$(n3) complex was more similar to the 9-aminoacridine compounds, as the RI of the 10th most intense damage site was 0.41.

Most damage sites consisted of a cluster of resolved fragments spanning approximately 3 or more bp, and the quoted bp position of damage refers to the most intense fragment. While ac-$PtenCl_2$(n3) shared a similar site of strong damage to cisplatin at bp 415−418, the most intense fragment occurs 3′ (toward the bottom of the gel) as compared to that of cisplatin by 1 bp (Figure 3). The polymerase approaches from the 3′ direction of the sequence, and for ac-$PtenCl_2$(n3), it pauses a base earlier at this site of damage. This is observed often for ac-$PtenCl_2$(n3) and never for $PtenCl_2$ and $PtenCl_2$(n2,3). This effect has been previously documented for phenanthridinium platinum complexes (*10*) and is probably due to the bulkier acridinecarboxamide platinum lesions causing the *Taq* DNA polymerase to halt before the platinum-induced lesion.

Time Course of the Reaction. Time course reactions of cisplatin, $PtenCl_2$, $PtenCl_2$(n2,3), ac-$PtenCl_2$(n3), and 9-aminoac-$PtenCl_2$(n2−5) have been determined in this study. A typical time course experiment was carried out at times $t =$ 0, 5, 15, and 45 min and 2, 6, and 22 h. A DNA sequencing gel for cisplatin, $PtenCl_2$, ac-$PtenCl_2$(n3), and 9-aminoac-$PtenCl_2$(n2) time course is shown in Figure 3. [It should be noted that for 9-aminoac-$PtenCl_2$(n3), the DNA has been overdamaged at longer time points (Figure 3, lanes 31−34)].

Line graphs showing the relative time course for cisplatin and the targeted platinum complexes are shown in Figure 4. The damage rates fall into two broad categories; those that damage at short incubation times, ac-$PtenCl_2$(n3) and 9-aminoac-$PtenCl_2$(n2−5), and those that damage at long incubation times, cisplatin, $PtenCl_2$, and $PtenCl_2$(n2,3). All complexes that damaged at short incubation times possessed an attached acridine moiety. A comparison of the time taken to reach half the maximum relative intensity indicates that ac-$PtenCl_2$(n3) and 9-aminoac-$PtenCl_2$(n2−5) reacted at least 10-fold faster than cisplatin and $PtenCl_2$. Ac-$PtenCl_2$(n3) exhibited a marginally faster rate of damage than the 9-aminoac-$PtenCl_2$(n3) complex, which is perhaps surprising as the more basic 9-aminoacridine is a better intercalating agent.

In each set of lanes, representing the time course of a single compound, 10 damage sites were analyzed, and the intensity values were normalized relative to the time point of maximum intensity. For each compound, it was observed that all damage sites exhibited the same rate of damage. Thus all damage sites are damaged at the same rate, and the damage rate is not sequence specific.

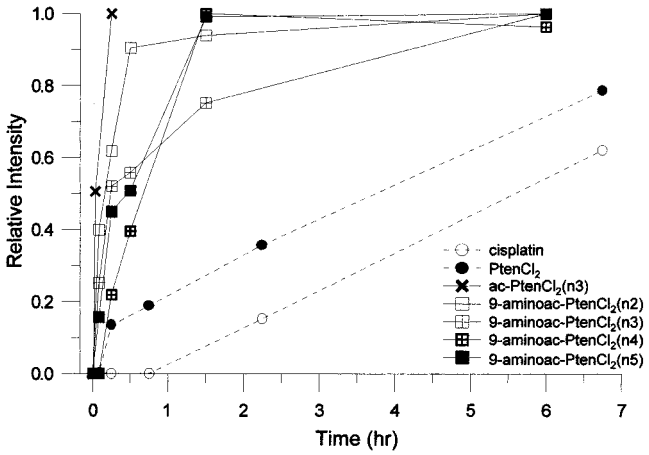

FIGURE 4: Line graph showing the time course of DNA damage. Compounds shown are cisplatin, $PtenCl_2$, ac-$PtenCl_2$(n3), 9-aminoac-$PtenCl_2$(n2), 9-aminoac-$PtenCl_2$(n3), 9-aminoac-$PtenCl_2$(n4), and 9-aminoac-$PtenCl_2$(n5). Each line represents the normalized relative intensity for each analogue, and each point is the average of five damage sites. Data were taken from Figure 3 and other comparable gels (not shown).

DISCUSSION

Sequence specificity and relative time course of DNA adduct formation of cisplatin and 12 related compounds were investigated using a plasmid system. A linear amplification/DNA sequencing technique was used to determine the precise sites of adduct formation, and densitometry was used to determine the degree of damage at each site.

Five groups of compounds were examined (Figure 1). The 9-aminoacridine platinum carboxamide complexes (group 2) showed more rapid rates of reaction and different sequence specificity as compared to cisplatin (group 1). The acridine platinum carboxamide complex (group 3) also had an accelerated rate of reaction but a similar sequence specificity as compared to cisplatin. The platinum complexes with an attached linker but no intercalating chromophore (group 4) had a similar rate of reaction and similar sequence specificity as compared to cisplatin. The 9-aminoacridines with attached linker but no platinum (group 5) did not damage DNA.

Taken together, these data reveal that the 9-amino substituent is responsible for the altered sequence specificity. Also the presence of an intercalating group results in an accelerated rate of platinum reaction with DNA. This study represents the first occasion that an altered sequence specificity has been convincingly demonstrated for a cisplatin analogue.

Sequence Specificity. Sequence specificities of cisplatin and related complexes, as determined in this study, are shown in Table 1. The sequence specificities of $PtenCl_2$ and $PtenCl_2$-(n2,3) complexes were found to be very similar to that of cisplatin, with runs of consecutive guanines being preferentially damaged. For Ac-$PtenCl_2$(n3), the major sites of damage were similar to cisplatin (runs of consecutive guanines), but there were numerous additional minor damage sites, as found previously for this compound (*7*). It is interesting to note that these minor damage sites are the major damage sites for the 9-aminoacridinecarboxamide platinum complexes.

The 9-aminoac-$PtenCl_2$(n2−3) analogues had a markedly differing sequence specificity as compared to cisplatin. Runs of consecutive guanines were not the most intense sites of damage, with 5′-tgAat-3′, agAgt, gaCct, caCga, tgCag, cgAgc, acGcc, and ctGca sites instead being prominent. All of these sites except the last two have purine dinucleotides present and probably involve purine N-7 intrastrand cross-links. The acGcc and ctGca sites could be GG interstrand cross-links. However, there is the possibility of unusual lesions not involving the N-7 of purines being significantly present. These could be caused by a novel reaction mechanism—direct displacement of chloride by a DNA nucleophile. These unusual sites are also present in the acridinecarboxamide ($n = 3$) platinum and also may result from a novel reaction mechanism.

There are a number of possible explanations for the altered sequence specificity of the 9-aminoacridine platinum complexes (reduced reaction at runs of consecutive guanines) as compared to cisplatin and the acridine platinum complex.

(1) As compared to the acridine analogue, the 9-aminoacridine analogues contain an extra amino group and are positively charged at pH 7.5. This extra functionality could provide new base pairing possibilities. This could result in a different pattern of DNA intercalation and DNA platination. The additional 9-amino functionality could also result in binding to bases other than guanine.

(2) The more rapid covalent reaction by the 9-aminoacridinecarboxamide platinum complexes suggests that the reaction is occurring directly from the intercalated state. The orientation of the carboxamide linker and platinum will be crucial in determining the interaction with DNA. Recent X-ray diffraction structures of 9-aminoacridinecarboxamide analogues (lacking platinum) complexed to DNA (*18, 19*) show that the carbonyl oxygen of the carboxamide linker forms a hydrogen bond to the protonated N-10 of the 9-aminoacridine, thus directing the side chain into the DNA major groove. This specific interaction cannot occur with the unprotonated acridinecarboxamide (because the 9-amino group is absent), so that orientation of the side chain (which bears the platinum complexes) is likely to be different. If the same structural features are found for the platinum complex, the orientation of the platinum group will be different for the 9-aminoacridine complex as compared to the acridine complex. Hence, the platinum group will be in a different position in the DNA helix and could react with a different sequence specificity. [See Adams et al. (*19*) for discussion of evidence that the carboxamide chain is in the major groove.]

(3) The fact that the greatest difference in sequence specificity (away from runs of guanines) occurred with the shorter chain length 9-aminoacridine platinum complexes suggests that the platinum atoms in more rigid structures have some difficulty in reaching these usually favored, most electronegative sites and thus are available to react at alternative ones. Footprinting studies of 9-aminoacridine-4-carboxamides (lacking platinum) have shown a preference for runs of consecutive guanines (*20*). This footprinting data would tend to confirm the importance of steric and positional factors. Until three-dimensional structures of the 9-aminoacridine and acridine platinum analogues bound to DNA have been determined, the precise reason for the novel sequence specificity will be unclear.

The novel sequence specificity of the 9-aminoacridine platinum complexes reported here is not due to the formation

of monofunctional adducts. The *Taq* DNA polymerase/linear amplification system has the ability to distinguish between mono- and bifunctional intrastrand adducts (*9*), in that monofunctional damage sites are displaced toward the top of the gel. This displacement was not observed for any of the group 2 compounds.

Previous attempts to alter the sequence specificity of cisplatin analogues have not succeeded. There is a report of a novel adduct by the cisplatin analogue AMD574 (*21*), but this is probably due to an artifact of the linear amplification system caused by a monofunctional adduct (*9, 22*).

The 9-aminoac-PtenCl_2(n2−5) analogues with short linker chain length exhibit more differing sequence specificity as compared to cisplatin than do the longer linker lengths. We have previously shown that linker chain length is an important factor in the interaction of *n*-bromoalkylphenanthridinium bromides and platinum phenanthridinium complexes with DNA (*10, 23*).

Time Course of Interaction with DNA. Since the biological site of action of cisplatin is thought to be DNA, the influence of DNA targeting on the time course of platinum adduct formation by ac-PtenCl_2(n3) and 9-aminoac-PtenCl_2(n2−5) complexes was investigated in some detail. Other studies have examined the kinetics of interaction of various cisplatin analogues (although not with attached DNA-binding moieties) with DNA and found increases in the rate of reaction as compared to cisplatin (*24, 25*). The mode of interaction of ethidium-tethered platinum complexes with DNA has been investigated (*26*).

In the experiments described in this paper, we observed that 9-aminoac-PtenCl_2(n2−5) and ac-PtenCl_2(n3) produced relative damage rates at least 10-fold greater than cisplatin and PtenCl_2, suggesting mechanistic differences between the targeted complexes and cisplatin (and PtenCl_2). We have previously hypothesized that the observed increase in reaction rate for platinum phenanthridinium complexes is due to a different reaction mechanism—direct displacement of chloride by the N-7 of guanine in DNA (*10*). The presence of an attached intercalator leads to an acceleration of the reaction rate. Hence, we conclude that 9-aminoacridine and acridine platinum carboxamides also have a different reaction mechanism to cisplatin—direct displacement by the N-7 of guanine. It is also interesting to note that all sites are damaged at the same rate. Hence, the acceleration of rate is not sequence-specific but affects all damage sites equally.

We also included control compounds to examine the effect of the intercalating moiety on the reaction with DNA. Compounds with an attached polymethylene chain but no intercalating chromophore (group 5) had a similar rate of reaction (and sequence specificity) to that of cisplatin. This indicates that the attached intercalator is responsible for the increase in rate of reaction with DNA. The metal-free 9-aminoacridine ligands (group 4) did not damage DNA, and hence the acridine intercalator does not have any effect on the passage of *Taq* DNA polymerase.

Evidence has been presented in this paper to indicate that targeting of platinum to DNA by its attachment to an intercalating chromophore results in a marked increase in the rate of platinum binding to DNA. 9-Aminoacridinecarboxamide platinum complexes also have an altered sequence specificity. The latter complexes also have antitumor activity in a tumor-bearing mouse model and activity against cisplatin-resistant cells (*27*). This could be due to the different sequence specificity of these analogues. The production of novel lesions in DNA may lead to evasion of DNA repair and increased cell toxicity. As a result, these compounds could form the basis of a new class of cancer chemotherapeutic agent.

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